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TRACE ELEMENTS IN FOOD

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by

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LONDON
CHAPMAN & HALL LTD.
37 ESSEX STREET W.C.2
1949

First published . . . *1949*

THIS BOOK IS PRODUCED IN COMPLETE
CONFORMITY WITH THE AUTHORIZED
ECONOMY STANDARDS

Catalogue No. 369/4
Printed in Great Britain by Butler & Tanner Ltd., Frome and London
Bound by G. & J. Kitcat Ltd., London. *Flexiback Binding*

PREFACE

THE term 'trace element' is usually applied in the biological sense to those elements which, in extremely small amounts, play some part in the nutrition of plants or animals. It may equally well be applied to the larger number which have for the food chemist a toxicological rather than nutritional interest, and the presence of which in food must, on public health grounds, be subject to close scrutiny and control.

There is for most trace elements a wide range between those quantities which are by common consent innocuous or even essential to health, and those which show signs of being injurious. To decide on the limits within this range to which foods should conform is not easy, and it is one of the trials of the food chemist that he is so often called upon for an expert opinion in the absence of any pronouncement, statutory or otherwise, by higher authority. He must therefore have as wide a knowledge as possible of all the questions involved ; the biochemistry, nutritional significance and toxicology of trace elements ; how far their presence in food is due to their being essential or occasional constituents of plants, or to the use of their compounds in agricultural practice ; the many ways in which they may gain access to food from manufacturing processes or commercial usage ; the bearing of existing Acts and regulations ; and finally the determination of trace elements in animal and plant tissues and in foods. In the following pages an attempt is made to give a comprehensive survey of the whole field.

I have included among trace elements those which may be present in the human body, and in most foods, in amounts up to 0·005 per cent. This brings in iron, which is not usually regarded as a trace element in the body but is certainly so in food.

Trace elements may be roughly grouped according to whether they are essential or non-essential to life, toxic or non-toxic in moderate excess, universally distributed or of casual occurrence. Of the twenty or so which occur commonly in foods about five or six only are known to be necessary to human or animal life (copper, zinc, iron, manganese, iodine, and to a limited extent cobalt). Three more (boron, molybdenum and silicon) are apparently essential only to plant life. Of the others a few occur spasmodically in plants (e.g. selenium), while a good many (lead, tin, arsenic, antimony, cadmium, nickel, etc.) gain access to food from industrial processes. Some of them are highly toxic, others are relatively harmless. Elements closely related chemically do not necessarily show any resemblance in their biological behaviour, toxicity or effects on food, and each of them must be considered separately according to its particular characteristics. The arrangement of the elements in the following pages depends partly upon their relative significance as constituents of food and partly on their chemical relationships.

The analytical sections are in the form of a general outline rather than a detailed laboratory guide ; a companion to, and not a substitute for, existing literature on the subject. Discussion is confined for the most part to salient points and principles, and for each element several alternative methods are reviewed and compared. Emphasis is laid on those suitable for general work rather than on specialised methods used in routine control.

My best thanks are due to Mr. W. A. Godby, A.R.I.C., for his invaluable help.

G. W. M-W.

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[COPPER]

COPPER as a trace element in food has received much attention in recent years, owing to its great activity as an oxidation catalyst and the important part which it plays in plant and animal life, even to the extent perhaps of being an essential element in almost all cellular respiration.

As a metal its properties of malleability, high heat conductivity and relative resistance to corrosion would make it ideal for the construction of food manufacturing plant and kitchen-ware, if it were not for the fact that it has acquired, rightly or wrongly, a bad reputation for toxicity. Hence its presence in food in any considerable quantity is looked upon with suspicion by public health authorities, and the use of its compounds for colouring food has been prohibited in this country.

In agriculture it is an important element in at least three entirely different ways: copper fungicides for fruit and vegetables, diseases of stock from lack of copper in pasture, and spoilage of dairy products by catalytic oxidation.

COPPER AS AN ESSENTIAL ELEMENT IN PLANT AND ANIMAL LIFE.

Enzymes. Copper is intimately associated with tissue oxidases, several of which appear to be copper-protein compounds. Among these are the polyphenol oxidases of potatoes (Kubowitz 1937), of mushrooms (Keilin and Mann 1938) and of tea (Sreerangachar 1943), the enzyme laccase (Keilin and Mann 1939) and the ascorbic acid oxidase of cabbage (Stotz and others 1937). Indophenol oxidase, which is concerned in cell respiration through its power of oxidising cytochrome

(p. 240), is probably also a copper compound (Keilin and Hartree 1938). Copper would seem, therefore, in common with iron, to be an essential element in cell respiration. According to the view that an enzyme consists of a protein combined with an active prosthetic group, the copper in these enzymes may be looked upon as the prosthetic group. When the copper of polyphenol oxidase is removed by dialysis the protein is inactive; addition of copper ions to the protein restores activity.

In enzymes containing copper or other metal as the prosthetic group it is the protein component which is specific and which determines the character of the enzyme. The catalytic activity of the enzyme is far greater than that of the metal itself and may be effective at dilutions at which the metal cannot be detected by any known chemical or physical methods (Keilin and Mann 1944).

Haemoglobin Formation. In the blood of vertebrate animals the conversion of iron stored in the liver into haemoglobin cannot take place unless traces of copper are present (Hart and others 1928, McHargue and others 1928). Rats kept on a diet of cows' milk, which is notoriously low both in iron and copper content, become anaemic and remain so even after addition of iron to the milk, but recover quickly with a daily dose of 0·01 to 0·025 mg. of copper (Hart 1929). Copper appears to be the only metal that can act as a catalyst in this process. The statements of some observers that manganese and other metals may have a similar effect in the conversion of iron into haemoglobin are due possibly to the difficulty of excluding minute traces of copper from experimental diets. Sufficient iron must of course be available to enable copper to be fully effective in the treatment of anaemia. The optimum proportion of iron to copper in the diet is given variously by different authorities as between 8 to 1 and 25 to 1 (Hart and others 1937). It may be that iron salts used in the treatment of nutritional anaemia often owe their efficacy to contamination with traces of copper.

Mann and Keilin (1938) have isolated from red blood corpuscles a blue crystalline copper-protein compound which they call 'haemocuprein', containing 0·34 per cent of copper, but whether this substance is an active agent in haemoglobin synthesis is doubtful. The amount of haemocuprein in 100 ml. of red blood corpuscles is 0·06 g., corresponding to 2 p.p.m. of copper. Tompsett (1934) and others have given similar figures (1·8 to 2·3 p.p.m.) for the copper content of human blood. The copper in blood and in tissues is more loosely bound than is the iron in haematin. It can, in contrast to haematin iron, be completely extracted with trichloracetic acid.

The suggestion has naturally been made that the action of liver extracts, largely used for the treatment of certain kinds of anaemia, may depend primarily upon their copper content (Hart 1929, Schultze 1932). This view is, however, not now accepted, and the action of these extracts is ascribed to a specific anti-anaemic substance or substances. Although copper is essential to the actual formation of haemoglobin, and probably also of the erythrocytes or red blood cells, lack of haemoglobin or of blood cells may be due to many causes beside copper deficiency.

Copper in other Biological Processes. Several other biological processes are activated by traces of copper. It is stated to be essential for the action of adrenalin (Handovsky 1934) and also for the production of toxins by *B. diphtheriae.* This bacillus thrives in concentrations of copper (2 to 8 p.p.m.) which would be toxic to other organisms (Leulier and Sedaillan 1930, Locke and Main 1930, 1931).

Some investigators have claimed that there is a marked parallelism between the contents of vitamin B and copper in various biological materials (Kerp 1933, Zondek and Bandmann 1933), but with more exact knowledge of the chemical nature of the different components of the vitamin B group it has not been shown that copper is essential to the action of any of them (cf. zinc and vitamin B_1, p. 109).

Copper is stated to have an effect on the amount of sugar in blood, in that it activates glycolysis. In diabetes it may act as an insulin sparer, and mild cases can be maintained on copper without giving insulin (Schnetz 1937). In rats suffering from nutritional anaemia, sugar feeding causes a sudden rise in blood-sugar, faster than in normal rats, and if 0·1 mg. of copper is given daily it reduces the rate (Keil and Nelson 1934). On the other hand, there is some evidence that in higher concentrations copper may inhibit glycolysis. Lipmann (1934) found that while 0·06 to 0·6 p.p.m. of copper greatly increased the glycolytic activity of a muscle extract, there was an inhibition of glycolysis if the copper concentration was raised much above 0·6 p.p.m.

Haemocyanin. In the blood of some invertebrates, notably crustaceans, gasteropods, and cephalopods, copper in the form of haemocyanin replaces iron. Haemocyanins from different sources may vary in composition and in the amount of copper they contain. In general, the copper content of haemocyanin is in the region of 0·2 to 0·4 per cent and that of the blood of crustaceans 50 to 60 p.p.m. (Guillemet and Gosselin 1932, Montgomery 1930), but the latter ranges from 35 to 237 p.p.m. in different species (Dhéré 1915). Haemocyanin appears to act as an oxygen carrier in invertebrate blood, one atom of oxygen being taken up for each atom of copper. It is blue when in the oxidised state but colourless when reduced.

Copper is easily split off from the molecule by slight acidification (pH 2·5) followed by dialysis (Barkan 1933, Roche 1930). The compound is probably a copper proteinate and is not constituted on the model of the haemoglobin molecule.

A few species have been found in which the blood pigments contain manganese, nickel or vanadium in place of iron or copper.

COPPER IN ORGANS OF THE BODY. The human liver contains 3 to 13 p.p.m., averaging about 5 p.p.m. (Herkel 1930, Tompsett 1934), and the other main organs from 2 to 4 p.p.m. The livers of newly born children may contain five to six times as much copper as in later life (Morrison and Nash 1930), possibly a provision of nature to enable blood formation to proceed in spite of the relatively low copper content of human milk (0·4 p.p.m.). The spleen of newly born children is also rich in copper (Lesné and others 1936). In animals the copper content of the liver varies greatly with different species, but with most of them it is much higher in the young than in the adult, calf liver, for instance, containing more copper (150 p.p.m.) than ox liver (20 to 80 p.p.m.).

In the human skeleton, the ribs contain from 4 to as much as 48 p.p.m., averaging 15 p.p.m., but the vertebrae only 2 to 5 p.p.m. (Tompsett). According to Chou and Adolph (1935) the whole body of an adult contains 100 to 150 mg. or about 1 to 2 p.p.m.

BIOCHEMICAL AVAILABILITY OF COPPER. Unlike iron which is of value in anaemia therapy only when it is in ionisable form, most of the naturally occurring salts and compounds of copper seem to be readily available for use by the organism. Schultze and others (1936) found that the copper of wheat germ, yeast and meat, copper aspartate, citrate, nucleinate, caseinate and pyrophosphate, the copper compound of cysteine, and cuprous mercaptide, were all readily utilised by anaemic rats in haemoglobin regeneration. An exception is cupric sulphide, owing presumably to its insolubility in the fluids of the digestive tract. In certain foods such as eggs it may occasionally happen that the copper present is largely in the form of sulphide and therefore inert (Sherman and others 1934). Copper in haemocyanin, the blood pigment of shell-fish, is readily available, but when in combination with the porphyrin complex, as in turacin (p. 10) and possibly in copper phyllocyanate (p. 24), it appears to pass through the alimentary tract without being absorbed (Schultze, Elvehjem and Hart 1934).

ABSORPTION, EXCRETION AND RETENTION. Absorption of toxic metals from the alimentary canal takes place chiefly by way of the portal vein to the liver, whence the absorbed metal may pass back into the intestine by way of the bile duct and thence to the faeces, or it may pass from the liver into the general circulation and be excreted eventually in the urine (cf. diagram on p. 70). Urinary excretion provides a means of assessing the amount of absorption as far as the general circulation is concerned, but the total amount of absorption from the alimentary tract is not so easily determined. According to Judd and Dry (1935) the bile contains a considerable amount of copper and may be one of the chief paths of excretion, with the result that much of the absorbed copper appears in the faeces. Thus in attempting to estimate the extent of absorption from the alimentary tract we encounter the difficulty, with copper as with many other metals, of distinguishing between that which passes through the tract unabsorbed, and that which is absorbed and subsequently excreted by way of the bile or other secretions or through the intestinal wall. The large amounts sometimes found in gall-stones (160 to 2,000 p.p.m.) presumably originate from copper in the bile (Schönheimer and Oshima 1929).

With copper, as with most other metals, it seems that a distinction must be drawn between the absorption of the small amounts normally present in food, and that of larger amounts which may approach the toxic limit. The larger the quantity ingested with food the smaller is the relative absorption, that is to say, absorption, although rising with increasing intake, is far from keeping pace with it.

The mean daily intake in food, when coppered vegetables are not eaten, may be taken as being about 2 mg. (p. 30). The normal urinary excretion of copper is given by Tompsett (1934) as 0·08 to 0·48 mg. daily, representing on an average about 15 per cent of the amount ingested, and Rabinowitch (1933) gives somewhat similar figures. Ross and Rabinowitch (1935) found an average of 0·16 mg. daily in the urine of children, which might represent about 10 per cent, or less, of the total copper ingested with food. Scoular (1938) found that with boys of 4 to 6 years the urinary excretion of copper was fairly constant at 4 per cent of that ingested.

When larger amounts of copper are consumed with food the amount in the urine rises slightly, but it seems that any excess of copper above the normal tends to be voided in the faeces rather than in the urine. Drummond (1925) found that in rats receiving comparatively large doses in the form of peas coloured with a copper salt, only 0·5 to 0·7 per cent of the copper was excreted in the urine. It is possible that the compound of copper with chlorophyll may not be broken down in the alimentary tract (p. 24) and that copper in other forms may be more readily absorbed. Neither Taylor, Long nor Chittenden, in experiments carried out in 1913 for the United States Department of Agriculture, could find any copper at all in the urine when coppered peas were eaten, but their tests were not as delicate as those now available.

Retention of copper in the body seems to depend in some degree upon intake. Figures given by Scoular (1938) and by Daniels and Wright (1934) for children taking in between 1 and 2 mg. of copper daily, indicate considerable retention, ranging roughly from 0·25 to 1 mg., and similar figures have been obtained by Leverton and Binkley (1944) for young women. Leverton and Binkley suggest that copper, like iron (p. 244), is tenaciously held by the body. It is not excreted through the intestinal wall, and loss of copper from the body is limited to urinary excretion and occasional haemorrhage. It is possible, however, as has been pointed out above, that even if copper is not excreted through the intestinal wall it may still pass into the intestine by way of the bile duct. Tompsett's work (1934) indicates that, of the copper normally present in food, the total amount excreted by adults in urine and faeces follows fairly closely the amount taken.

When ingested in moderate excess with food, copper does not appear to accumulate in the body. Taylor (1913) found that when 15 to 25 mg. were consumed daily for two months in the form of coppered vegetables, about 12 per cent of the total copper ingested was temporarily retained in the liver, from which it was slowly eliminated by way of the faeces. Long (1913) found that copper retention was almost nil. With still larger doses, however, and possibly also in certain pathological conditions such as haemochromatosis, copper undoubtedly accumulates in the liver, as has been shown repeatedly in animal feeding experiments (p. 11).

MINIMUM REQUIREMENTS OF COPPER. Owing to the uncertainty attaching to the results so far obtained for absorption and retention of copper, it is difficult to say what is the minimum amount required to balance wastage and excretion and thus to maintain the necessary concentration in the body. Some authorities have given the requirement for a man as 1 to 2 mg. daily (Chou and Adolph 1935) and much the same for a child, about 0·1 mg. per kilogram of body weight or 2 mg. daily for a child of about 45 lb. Scoular (1938) gives the requirements for boys of 4 to 6 years as 0·06 to 0·08 mg. per kilogram. If these estimates are correct they would indicate that many people receiving normally about 1·7 mg. of copper daily in food (p. 30) are living on the border-line of copper deficiency. Hawksley (1934), indeed, considers that one case in twenty of nutritional anaemia in infancy is due to copper deficiency, and Innes (1936) draws attention to a condition in children which closely resembles the disease of lambs known as 'swayback' and associated with lack of copper in the blood. If, on the other hand, Leverton and Binkley are right in saying that copper is lost only through the kidneys and by haemorrhage a very small daily intake may be enough to maintain the body store at the necessary level. Tompsett, from the results of experiments on the normal intake and excretion of copper, suggests that the daily requirements for adults may be in the region of 0·6 mg., a quantity amply supplied by practically all diets.

Experimental rats can be kept in good health on a diet containing only 0·01 mg. a day (Cunningham 1931), or 1 p.p.m. of their food assuming a daily consumption of 10 g. of food. Weight for weight this is of the same order as the normal intake for a man. Schultze and Elvehjem (1933) say that 0·005 mg. is enough.

In pigs suffering from nutritional anaemia 5 mg. daily produced rapid recovery, but this is not necessarily the minimum requirement in normal health. It is stated that a practical laying ration for hens contains 0·5 mg. of copper (Schultze and others 1936). These amounts in relation to body weight are in excess of the average human intake.

Some of the mineral constituents of vegetables, including copper, iron and manganese, are lost when they are boiled in water (Culp and Copenhaver 1935), but since, in an average diet, vegetables contribute only one-fiftieth of the total amount of copper consumed, the loss of copper is negligible.

COPPER DEFICIENCY EFFECTS IN CATTLE AND SHEEP. Domestic animals have not the varied diet available to man, and cattle and sheep especially are largely dependent on mineral elements taken up by pasture over a very limited area. It is not therefore surprising to find that on some soils lack of sufficient copper in pastures is responsible for diseases of stock. ‘ Salt-lick ’ sickness of cattle in the sandy districts of Holland and on land reclaimed from the Zuider Zee (Brouwer and others 1938) is an anaemia, accompanied by scouring, apparently due to lack of copper (Sjollema 1933, 1938). The copper content of hay from these districts is only 1 to 3 p.p.m., on the dry weight, in place of an average of 7·5 p.p.m. in unaffected areas. The blood of cattle suffering from this ailment contains only one-third of the normal amount of copper. Dosing with copper salts or removal to pastures with a high copper content effects a rapid cure. A similar condition is found in cattle on the sandy and peaty soil of Florida (Neal and others 1931), in New Zealand (Cunningham 1944) and in Aberdeenshire (Jamieson and Russell 1946). In parts of Australia lack of copper causes enzootic ataxia (‘ gingin ’ disease) in sheep and cattle. The livers of affected sheep contain only 4 to 8 p.p.m. calculated on the dry weight in place of a normal 120 to 360 p.p.m., and the blood also has only a fraction of the normal copper content (Bennetts 1937, 1941, 1942). One of the earliest and most obvious symptoms is ‘ stringy ’ wool. It is probable that copper deficiency may be partly responsible for ‘ coast ’ disease of sheep in Australia (p. 286), a disease primarily due to cobalt deficiency (Marston and others 1938). Lack of copper in the blood seems also to be associated with ‘ swayback ’ of lambs in North Derbyshire (Dunlop and

Wells 1938, Shearer and McDougall 1944), although the possibility that lead poisoning plays some part cannot be excluded (Watson 1944).

It may be noted that in one district of England it has been established that a cattle sickness is due to poisoning by molybdenum in pasture (p. 470). This condition is curable by giving the animals copper sulphate, but is not due primarily to copper deficiency.

PIGMENTATION. It has been found that loss of pigment in the hair of black rats, caused by rapid growth on certain diets, is cured by giving them copper (Gorter 1935). The amount required to restore the pigment is 0·07 to 0·24 mg. daily, which is considerably higher than that required for the cure of nutritional anaemia. Japanese investigators have found that black hair contains much more copper, 6 to 8 p.p.m., than light-coloured or white hair (Sarata 1935). The copper content decreases as hair becomes grey and less melanin is produced (Yoshikawa 1937). According to Unna and others (1941) deficiency of pantothenic acid may also be a factor in causing loss of pigment in rats' hair. Whether copper is essential to the production of melanin, and whether it plays any part in skin pigmentation, does not seem to have been established. It has been found associated with the pigment cells of the retina of the eye, to the extent of 0·003 to 0·008 μg. in the retina of the ox (Baroni 1937). The dried ink of *Sepia officinalis*, the common cuttlefish, yields 1·8 per cent of ash containing over 60 per cent of copper which suggests that copper is an essential constituent of this pigment (Giuliani 1938). Turacin, a pigment found in the feathers of certain birds, contains 7 to 8 per cent of copper and appears to be a compound of uroporphyrin, with the formula $C_{40}H_{34}O_{16}N_4Cu$. It is thus related to haemoglobin and chlorophyll, both of which contain a porphyrin complex or aggregate of four substituted pyrrol groups (Fischer and Hilger 1924).

TOXICITY OF COPPER. Copper salts are strongly astringent and irritating to the stomach. When administered as sulphate the astringent dose for man is given as 16 to 32 mg.

of copper and the emetic dose 80 to 160 mg. If copper is absorbed into the blood in considerable amounts it acts as a haemolytic agent.

In common with other heavy metals copper may, in certain circumstances, combine with the thiol group and may thus interfere with enzyme systems which depend upon this group for their operation. McCance and Widdowson (1946) have shown that when 2:3-dimercapto-propanol is injected intravenously the excretion of copper in the urine is increased ten- or twenty-fold, indicating that copper has combined with the dithiol to form a soluble compound readily excreted by the kidneys. Barron and Kalnitsky (1947) discuss the possibility that the toxic action of many heavy metals may depend upon their ability to combine with thiols in the body.

The question whether or not chronic copper poisoning can be caused by the repeated ingestion of small doses has not been answered satisfactorily. Rats fed on food containing 500 p.p.m., equivalent to an intake of about 5 mg. of copper daily, have not shown any evidence of poisoning (Coulson and others 1934), but above 500 p.p.m. toxic effects begin to appear (Boyden and others 1938). The liver shows an increase of 14 to 20 times the normal amount of copper, and there is an increase of about 40 per cent in the copper content of the spleen and 20 per cent in that of the blood. According to Datta (1941) rats fed on whole wheat and milk with 4 mg. of copper daily (as sulphate) grew normally, but with 6 to 9 mg. growth was adversely affected. Cunningham (1931) found that rats could ingest 7·5 mg. of copper daily in the form of sulphate or acetate without effect on growth or reproduction, but that when the copper was given in the form of basic acetate (' verdigris ') reproduction was stopped. It is difficult to account for this. Becker and others (1938) found that copper sulphate fed to cattle daily for some months at the rate of 5·9 g. per 1,000 lb. live weight did not hinder growth in one instance, but that in another animal 7·8 g. per 1,000 lb. live weight proved toxic. Sheep appear to be very susceptible to the action of copper (Rees 1933), and there are several instances on record of their being killed by grazing in fields contaminated from smelting works or in orchards where much spraying has been done. The livers of such sheep are stated to be heavily pigmented and to contain considerable quantities of copper (Beijers 1932, Bisset 1934). One gramme of copper sulphate given daily for one or two months will kill sheep (Hopkirk 1938–39).

According to Mallory (1921, 1925, 1931) copper poisoning may cause the disease known as haemochromatosis, characterised by pigmentation of the liver, due to haemolysis of the red blood corpuscles, associated with necrosis of liver cells, cirrhosis and diabetes. The pigmentation may affect other parts of the body, including the skin, whence the disease is sometimes known as ‘ bronze diabetes ’. In haemochromatosis the copper and iron contents of the liver are increased to many times their normal value (Funk and St. Clair 1930, Ramage and Sheldon 1935). Mallory's conclusions were apparently based on experiments in which rabbits received comparatively large amounts of copper with their food, 100 to 200 mg. daily. Hall and Mackay (1931) also fed rabbits upon food containing 2,000 p.p.m. of copper and produced pigmentation of the liver and in some instances cirrhosis and cell necrosis. On the other hand, Mallory's results were questioned by several workers who were unable to produce cirrhosis of the liver in rabbits by prolonged administration of copper. In some instances pigmentation resulted but no cirrhosis (Oshima and Siebert 1930, Schindel 1931) ; in others no effect was observed (Flinn and von Glahn 1929, Polson 1929). It is significant that Mallory in reply says ‘ it cannot be too strongly emphasised that a sufficient dose of copper must be administered to produce our results. If a certain minimum is not exceeded the animals can handle it with no injurious effects’; and again, ‘the action of copper in small amounts over a long period of time destroys such an insignificant number of erythrocytes each day that it is impossible to detect such a loss. It was not until we carried out our acute poisoning experiments that the action of copper on the blood was revealed.’ In other words, Mallory's observations were made on animals suffering from acute copper poisoning and cannot be taken as an indication of the existence of chronic poisoning from repeated ingestion of small quantities. There is no doubt that in haemochromatosis of the liver and also in ordinary cirrhosis, such as that due to alcoholism, the copper and iron content of the liver may be raised to many times the normal, but this may be the result of derangement of metabolism from other causes (cf. Sandberg and others 1942), and there is no evidence that ingestion of copper within reasonable limits can produce this condition in man or that when present in food in moderate amounts it can act as a haemolytic agent.

Incidentally, experiments with rabbits are affected by the fact that these animals practise faecal refection on a large scale, and some or all of the copper consumed may pass at least twice through the animal's body (Taylor 1939, Eden 1941). This should not, however, affect the ratio between the copper ingested and that finally excreted.

Copper strongly inhibits bacterial growth, and if present in too large amount in the alimentary tract might well affect bacterial action. Recent work has indicated that bacterial synthesis in the tract may be of significance in nutrition, and the possible effect of copper and other heavy metals, if not immobilised as sulphides, must be borne in mind.

Lower animals are much more sensitive to copper. It is possible to stop the growth of algae in water by adding 0·1 p.p.m. without harming fish (Czensny 1934), but 0·25 p.p.m. of soluble copper may be toxic to trout (Embrey 1917). Some coarse fish will stand as much as 2 p.p.m. Tadpoles are affected by water contained in a copper vessel (Schwaibold and Fischler 1931). According to Jones (1939) copper and other heavy metals affect fish by asphyxiation due to precipitation of gill secretions. Sea-water contains 0·01 p.p.m. of copper (Atkins 1932), and most marine molluscs will not tolerate more than 0·1 or 0·2 p.p.m. On the other hand, species living in estuary water liable to copper contamination, for instance oysters and mussels, seem to tolerate it more readily. Oysters especially may take up relatively large amounts. Possibly this is the result of efforts to filter out and immobilise dissolved and suspended copper from the water before it reaches the more sensitive parts of the organism. It has been suggested that there is a positive correlation between the copper content of estuary water and the 'setting' of oysters, i.e. the action of oyster larvae in cementing themselves to a clean surface, and that the optimum copper concentration for this is 0·5 to 0·6 p.p.m. (Prytherch 1931, 1934).

COPPER IN SOILS AND IN PLANTS. Traces of copper are apparently essential for plant growth, in amounts from 0·05 to 0·10 p.p.m. of the soil moisture or root medium (Lipman and Mackinney 1931, Sommer 1931). The amount present in normal soils varies widely from about 2·5 to 20 p.p.m. and in some instances as much as 50 p.p.m. (Greaves and Anderson 1936, Manns and Russell 1935, Maquenne and Demoussy 1919). Still higher figures have been recorded in Italy. It does not follow that all of this is available to plants (Rademacher 1936). Some of it may be fixed by humus components or immobilised in the form of cupric sulphide by hydrogen sulphide produced by bacterial action. It is suggested (Mulder 1939) that this happens in many moorland soils and in soils recently reclaimed from the sea, as in parts of Holland where the available copper is less than 2·5 p.p.m. In such soils various plant diseases occur such as 'wither tip' of apple trees (Dunne 1938), exanthema of pears (Oserkowsky and Thomas 1933), chlorosis of beet and other plants (Roach 1936, van Schreven 1936), 'die-back' disease of citrus trees (Stokes 1935) and a disease of oats in which they develop white paper-like tips on the leaves and loose white glumes (Arnd and Hoffmann 1937, Hieltjes 1937). These are due apparently to copper deficiency, and the plants affected usually contain less than 1 p.p.m. of copper in the dry material. They respond to treatment with fertilisers containing copper. In the Everglades district of Florida tomatoes and other crops can hardly be grown unless the soil is treated with copper sulphate (Schreiner 1930). Where a soil is found to be deficient in copper the application of 25 to 50 lb. of copper sulphate per acre is usually enough to restore it to fertility (Manns and Russell 1935). This would correspond to about 4 to 8 p.p.m. of copper in the top 8 inches of soil.

Primarily the need for copper in plants is no doubt due to its being an essential constituent of many plant oxidases. Whether it plays any further part either as a plant stimulant or as an essential plant food is not known. Leaves contain more copper than roots, which suggests that it may be necessary for respiration or photosynthesis (Orth and others 1934, van Schreven 1936) ; deficiency has been associated with a reduced rate of photosynthesis. Copper undoubtedly assists plant growth by its toxic action on bacteria and moulds (Munerati 1935) and may also act as a catalyst in promoting the oxidation of ferrous salts, which are toxic to plants, to ferric salts which are relatively non-toxic (Maquenne and Demoussy 1920).

Crops appear to take up a little more than the normal amount from soils rich in copper, but the increase in total copper content is not great. Mestre in 1890 found 2·5 p.p.m. in potatoes grown in coppered soil. Quartaroli (1929) found that even in soils containing ten times the average quantity of copper the amount taken up by plants was only slightly increased. Lettuce and spinach grown in soil to which copper has been added have shown an increase of copper in the ash (Miller and Mitchell 1931). Elvehjem and Hart (1929) found that on a plot treated with copper sulphate at the rate of 50 lb. per acre lettuce showed a small but definite rise in copper content, but in soil treated with 500 lb. of copper sulphate per acre the increase was 148 per cent. Greaves and Anderson (1936) found that in wheat, barley and oats grown on different soils with copper contents varying from 4 to 51 p.p.m., copper in the grain ranged from 5·6 to 16·7 p.p.m., but bore no relation to the copper content of the soil.

In high concentrations copper has a toxic effect, 60 p.p.m. in water culture having been found to interfere seriously with germination of seeds. The point at which copper in soil begins to be toxic to such crops as spinach and turnips has been given as 1,250 lb. of copper sulphate per acre (Beaumont and Holland 1935) or roughly 200 p.p.m. of copper in the top 8 inches of soil. In highly calcareous soils, which favour the formation of insoluble copper salts, this figure may be much higher.

COPPER FUNGICIDES. Copper compounds are widely used as fungicides in agriculture. In these the metal is usually in an insoluble form as basic copper sulphate (Bordeaux mixture), oxychloride, salicylanilide, etc. (Read 1935), and the efficacy of the spray depends largely upon its degree of dispersion (Holland and others 1929). According to Dubacquié (1934), mould spores have an acid reaction and dissolve copper from the spray residue.

Residual copper remaining on sprayed fruit is usually of the order of 1 to 3 p.p.m., but occasionally much higher amounts are found. Thus at the East Malling research station in Kent, loganberries which had been sprayed 15 days before picking contained 10 p.p.m. of copper (Shaw 1936), and tomatoes have been found with as much as 40 p.p.m. With grapes most of the copper remaining on the fruit after spraying is retained by the skins and lees and does not get into the wine (von der Heide and Hennig 1933, de Prado 1937).

When copper spraying has been regularly carried out in vineyards and orchards the soil may contain comparatively large amounts, up to several hundred parts per million, without any toxic effect on the vines or fruit trees (Maquenne and Demoussy 1919). This may be due either to the fact that copper from sprays stays for the most part in the upper 6 or 8 inches of soil and does not penetrate readily to the main roots of vines or fruit trees, or to the copper being relatively insoluble and non-available, especially in calcareous soils. Thus apples show no increase whatever in their normal copper content (about 0·5 p.p.m.) when grown on such soils, while pears show an increase only from 0·8 to 1·8 p.p.m. (Jones and Hatch 1937). The effect on crops in general of an excess of copper in soils has been discussed in the preceding section.

An interesting application of the fungicidal action of copper is the use of copper sulphate in the cold storage of pears (Tiller 1934). If the pears are wrapped in paper containing 5 per cent of copper sulphate the spread of *Botrytis* is markedly reduced. In this connection reference may be made to the somewhat similar use of paper wrappings impregnated with iodine for prevention of mould on grapes (p. 325).

To bacteria in water copper is toxic in concentrations higher than 5 p.p.m., but *B. coli* and *B. diphtheriae* seem to be particularly insensitive to it (Tammann and Rienäcker 1928, cf. p. 3).

CORROSION OF COPPER BY FOODS. Copper, if kept brightly polished and free from oxide film, is not readily attacked by foods. As metal it is more resistant to fruit juices than aluminium, monel metal, nickel, bronze or tin (Mrak and Cruess 1929), but in practice it is impossible to prevent the formation of a film of oxide which is more easily dissolved. If copper surfaces in contact with fruit juices are exposed to air, oxidation quickly takes place. It is stated that the presence of iron in fruit juices greatly increases the corrosion of copper (Kotlyar and others 1935). On the other hand, copper often becomes coated with a protective film which reduces corrosion. In wine-making plant, for instance, a protective layer of relatively insoluble copper tartrate may be formed (Hagues 1931), and in breweries corrosion of copper is greater when the surface is scoured than when it is allowed to become covered with a film (Matthews 1929).

In certain foods copper appears to dissolve more readily than other metals. According to Tranent (1935) glucose syrup is one of these, copper immersed in it for three weeks losing more weight than zinc, tin, aluminium or even iron.

Copper is electrochemically cathodic to many other metals, so that when it is in contact with one of these (e.g. tin, lead, nickel, iron, zinc, or cadmium) the tendency is for that metal to dissolve rather than copper and for any copper in solution to be precipitated. The rate of solution of the metal depends to some extent on the position it occupies in relation to copper in the electromotive series and the consequent intensity of local electrolytic action. Tin lies close to copper in the series, and the tinning of copper in food plant or appliances affords good protection from corrosion. It is only when the tin coating becomes dissolved or worn off in patches that serious corrosion of the copper can occur.

When milk containing copper is brought into contact with nickel, an exchange may take place between copper and nickel, the former being precipitated and the latter going into solution (Fink and Rohrman 1932).

Red brass containing 85 per cent of copper and 15 per cent of zinc withstands corrosion better than copper and is claimed to be superior to copper, nickel and aluminium for cooking vessels (Järvinen 1923). Yellow brass with 60 to 67 per cent of copper and the remainder zinc is more readily attacked and gives up zinc to foods (cf. Haase, p. 114).

COPPER IN DAIRY PRODUCTS

Milk. Cows' milk contains normally about 0·15 to 0·20 p.p.m. of copper and occasionally 0·4 to 0·5 p.p.m. Spring milk may often contain about twice as much as winter milk. The amount found in milk does not seem to depend upon that in the cows' food (Quam and Hellwig 1928). Cows have been given additional copper to the extent of 100 g. in 40 days without any increase in the copper content of the milk (Liebscher 1937). Human milk contains rather more copper, 0·4 to 0·6 p.p.m. (Broek and Wolff 1935, Zondek and Bandmann 1931). It is curious that milk which is the sole food of the young mammal during the first few weeks of life, at a time when blood formation is at its maximum activity, should be so poor in copper. Apparently the copper requirements at this period are met by the considerable store of this element accumulated in the liver during foetal life, a store which is gradually depleted until the young animal is weaned from a milk diet and begins to receive an adequate amount of copper in its food. Similar conditions obtain with iron. In young pigs the rate of growth is so rapid that the natural stores of iron and copper are not large enough and have to be artificially supplemented.

Pasteurised milk frequently contains about twice as much copper as raw milk owing to its coming into contact with copper or brass fittings, and dried milk up to 2 p.p.m. partly owing to the concentration of solids on drying (Schwarz and Fischer 1936). Dried milk prepared in copper plant has been found to contain from 4 to 11 p.p.m. which caused it to develop a rancid tallowy taste, owing to catalytic acceleration of the normal process of fat oxidation in presence of air ; Conn and others (1935) record as much as 17 p.p.m.

The solubility of copper in milk is greatest at 70° to 80° and decreases at higher temperatures (Gebhardt and Sommer 1931, Quam 1929). It is favoured by the presence of oxygen, and possibly the lessened solubility at temperatures above 80° is due to loss of dissolved air from the milk (Miscall and others 1929).

If the copper content of milk exceeds 1·5 p.p.m. a tallowy taste develops in a short time. According to Davies (1933), even 1·5 p.p.m. in milk kept at 0° to 5° is sufficient to taint it in 24 hours. This quantity will also quickly spoil cream (Sampey 1939). The use of old tin-plated copper coolers often gives a taint to milk. Nothing is known of the mechanism of the action of copper on milk except that it is closely associated with the fat. Homogenisation, whereby the fat is broken up into much smaller globules, prevents it to some extent (Ross 1937). Copper acts presumably as an oxidation catalyst ; complete exclusion of air diminishes the effect but does not stop it entirely (Ritter and Christen 1935). Normally the copper content of milk, if off-flavour is to be avoided, should not exceed 0·5 p.p.m. The average for processed milk as bottled is about 0·2 p.p.m.

Copper in milk may give a blue colour with hydrogen peroxide and *p*-phenylene diamine similar to that given by peroxidase (Storch's reaction) even though the milk has been heated, but the colour takes some time to develop (Orla-Jensen 1932) and is not quite the same hue as that given by peroxidase.

The starch-iodide test for hypochlorite added to milk as a preservative is not trustworthy if the milk contains more than 2·5 p.p.m. of copper (Hauser and King 1945).

Copper has the effect of prolonging the reduction time in the methylene blue test (Ritter 1935). This is not due to its bactericidal properties, since Wilson (1935) has shown that the bactericidal action of copper in water can be completely prevented by the addition of 0·5 per cent of milk. It is more probably due to the action of copper as an oxidation catalyst counteracting the reducing effect of the bacteria present. One would expect therefore that high-grade milk, comparatively free from bacteria, would be more easily tainted by copper than low-grade milk, in which the oxidising tendency of copper is to some extent neutralised by reducing bacteria. A rapidly growing microflora keeps the oxidation potential in the milk so low that oxidation of the fat cannot occur. In the United States it has been found that Grade A milk has occasionally an unpleasant oxidised flavour, and dairymen are said to have countered this by inoculation with lactic bacterial cultures. It has been suggested that this flavour may be due to the action of specific bacteria, but it seems more likely to be the result of contamination with copper (*Food*, Sept. 1940; cf. also Tracy and others 1933).

Many different reducing substances have been tried as anti-oxidants in milk powder and other fatty foods. Organic gallates in particular are very effective in preventing the oxidation of fat in milk powder by traces of copper.

According to Gould (1941) the cooked flavour of heated milk is due to sulphides and sulphydryl compounds derived from proteins. Copper hinders the development of the cooked taste and will remove it to some extent when formed.

Cheese. The copper content of cheese varies widely according to the extent to which copper vessels or fittings are used in cheese-making. Practically all of the copper in milk is precipitated with the curd and is found in the cheese. Cheese made in a copper vat may contain as much as 18 p.p.m. (Davies 1931). Krenn (1938) states that cheese curd detaches itself readily from the surface of a copper vat and is comparatively free from whey, while it adheres to the surface of a stainless steel vat and is difficult to work unless the metal has previously been greased. Traces of copper cause a pronounced discoloration of sour-milk cheese ; Schwarz and Fischer (1936) found that in a cheese of this type made in the Harz mountains discoloration was apparent with more than 2 p.p.m. and was intense with 18 p.p.m.

Butter. Copper content is an important factor associated with keeping quality in butter (Loftus-Hills and Scharp 1934). The maximum which should be allowed in New Zealand butter is given as 0·2 p.p.m., but this is often exceeded (Singleton 1937). Butter containing more than 0·3 p.p.m. does not keep well (Horral and Epple 1939). One part of copper per million can produce a fishy flavour in less than three days, even in cold storage, and a tallowy flavour in about four days. In seven days the butter is rancid (Davies 1937). With 2 p.p.m. a fishy flavour appears in 18 hours. Butter has been found to contain as much as 8 p.p.m., which quickly gives it a tallowy taste (Harvey 1939).

Butter made from pasteurised cream often has a high copper content, and it is important that cream for butter making should not be allowed to come into contact with this metal or its alloys.

Ice-cream. Copper contamination can cause an off-flavour in ice-cream, especially strawberry ice-cream, and the metal should be kept out of ice-cream plant and utensils (Tracy and others 1934). If the copper content is not more than 0·8 p.p.m. the taste is not affected (Dahle and Folkers 1932). By adding 0·5 per cent of finely ground oat flour to an ice-cream mix containing 2 p.p.m. of copper before pasteurisation the onset of oxidised flavour may be prevented or considerably delayed. The addition of 2 per cent of oat flour to a 40 per cent cream kept in cold storage for about three months and then used for making strawberry ice-cream is stated to have improved considerably the taste of the product (*Food Trade Review*, Nov. 1939). The action of the oat flour is presumably that of an anti-oxidant and is similar to that of many other types of anti-oxidants in preventing or delaying the onset of rancidity in fats.

COPPER AND VITAMIN C. Traces of copper in milk and in fruit and vegetable juices tend to oxidise and destroy vitamin C, probably by activation of ascorbic acid oxidase which, according to Stotz (1937), is itself a copper-protein compound. If milk is pasteurised in copper vessels it may lose nearly all of its ascorbic acid, whereas aluminium and nickel have no such action (Schieblich 1932, Schwartze and others 1931). Ascorbic acid is autoxidisable in alkaline but not in acid or neutral solution unless a catalyst is present. Traces of copper cause rapid oxidation at all pH values between 2 and 10, the maximum effect being at pH 7·0, the neutrality point. This catalytic action is perceptible with as little as 0·05 p.p.m. of copper. It is completely stopped by 0·06 p.p.m. of potassium cyanide and retarded by amino-acids, which form complex copper salts (Barron and others 1936), and by several other constituents of food such as thiol compounds (glutathione), certain purines and creatinine (Giri and Krishnamurthy 1941). Pyro- and metaphosphates also act as inhibitors, possibly owing to copper being precipitated as phosphate and rendered inactive. In extracting vitamin C from plant materials the addition of 2 per cent of pyro- or metaphosphate prevents oxidation of ascorbic acid by copper present in the material or in the distilled water or reagents used (Mack and Kertesz 1936). Thio-urea is another very effective anti-oxidant, since it combines with and de-ionises copper (Kawerau and Fearon 1944). It is reputed to be somewhat toxic and to affect the secretions of the thyroid gland (cf. p. 321). According to Mapson (1941) fluorides, which inhibit many reactions of metals by combining with and immobilising the metals, have no effect upon the oxidation of ascorbic acid by copper, but other halide salts inhibit it.

One of the objections to the use of copper salts for greening canned and bottled vegetables (p. 24) is the loss of vitamin C which takes place during the process. Schmidt-Nielsen and Yri (1936) found that in vegetables kept at 37°, 88 p.p.m.

of copper oxidised 200 p.p.m. of ascorbic acid in 1½ hours. Under reduced pressure oxidation was retarded. Chlorophyll itself also retards oxidation by combining with copper (p. 24).

Copper promotes the oxidation of sulphurous acid when this has been added as a preservative to wines, fruit juices, etc.

COPPER AND FERMENTATION. The statements of different investigators as to the effect of copper on fermentation are somewhat contradictory. Small amounts, up to 0·1 p.p.m., in fermenting liquids stimulate growth and cytochrome formation in yeast (Elvehjem 1931). Larger amounts inhibit growth, but the presence of much protein and non-sugar extract tends to inactivate copper. Inhibition of fermentation seems to depend upon the amount of ionised copper present in solution. Koch (1935) found that German beer worts contained 0·3 to 0·9 p.p.m., some of which was precipitated during fermentation : 1·5 p.p.m. of active soluble copper affected fermentation. Guillemet (1936) found that when copper sulphate was gradually added in increasing amounts to a glucose solution containing actively fermenting yeast the rate of fermentation did not begin to fall until the solution contained 0·5 p.p.m. of copper. With further additions of copper sulphate the production of alcohol gradually decreased and stopped when 2 p.p.m. of copper was present in solution. Most of the added copper was taken up by the yeast which at the start contained 30 p.p.m. in the dry substance. At 1,300 p.p.m. (dry substance) fermentation was checked, but the yeast continued to adsorb copper up to 2,500 p.p.m. At this stage the yeast could not form alcohol from glucose, but could still do so from hexose diphosphate. On the other hand, van Laer (1934) found that copper was not very toxic to yeast in high temperature (25°) fermentation and yeast could become acclimatised to it. Fermentation was not appreciably affected even when the yeast contained 0·95 per cent (9,500 p.p.m.) of copper in the dry substance.

Molasses ordinarily contain from 9 to 26 p.p.m. of copper. The amount in the wort as diluted and set for fermentation would be about one-fifth of this. Fermentation is not affected until the copper content of the molasses exceeds 25 p.p.m., or 5 p.p.m. in the wort as set for fermentation (Owen and Calma 1930).

Clearly the effect of copper depends greatly upon the nature of the medium and the conditions of fermentation. Different strains of yeast differ in their sensitivity to copper and can become partly acclimatised to it by repeated cultivation in media containing copper. Yeast continuously removes copper from solution, which tends to keep it below the point at which fermentation is inhibited. The yeast itself may thus adsorb a relatively large amount.

In wine most of the copper derived from the spraying of grapes with Bordeaux mixture remains in the lees or is removed by yeast during fermentation (Mathieu 1934).

GREENING OF VEGETABLES. Tschirch (1893) ascribed the action of copper in the greening of vegetables to the formation of copper phyllocyanate, a body allied to chlorophyll, and gave it the formula $(C_{24}H_{27}N_2O_4)_2Cu$. He found that this compound was insoluble in dilute acetic and hydrochloric acids and not easily attacked by peptic or pancreatic digestion *in vitro*, but that in dogs and rabbits it was digested in the alimentary tract and the copper behaved as if it had been administered in the form of a more soluble salt. Other investigators (Long 1913) have claimed that owing to its insolubility in acids copper phyllocyanate resists digestion and that the copper is not absorbed. Drummond (1925) found that in rats fed on coppered peas and receiving 1·5 mg. of copper daily per 100 g. live weight, the proportion appearing in the urine was only 0·5 to 0·7 per cent of the amount ingested. In Drummond's experiments, however, it would appear that the amount of copper in the peas was much larger than that required to combine with the chlorophyll and that most of it was present in the form of copper protein.

Chlorophyll is a compound of magnesium with a porphyrin (tetrapyrrol) molecule, and it is probable that in coppered vegetables copper replaces magnesium, forming a compound analogous to haematin, which is an iron porphyrin. The iron of haematin is not absorbed in the alimentary tract, and copper phyllocyanate may behave similarly. Cooking tends to break down the haematin molecule and to make its iron available, but copper phyllocyanate is apparently more resistant to cooking, and coppered vegetables keep their colour when boiled. Long (1913) was of opinion that the copper chlorophyll complex was remarkably stable even after cooking. The stable phthalocyanine dyestuff Monastral Fast Blue B is somewhat similar in constitution, being a compound of copper with four *iso*-indole (benzene pyrrol) rings.

The amount of copper introduced into vegetables in this process varies considerably. For most green vegetables 50 p.p.m. are sufficient to combine with all the chlorophyll, but samples have been found to contain 150 to 200 p.p.m. and in exceptional instances as much as 350 p.p.m. The usual amount is 2 gr. of crystallised copper sulphate per lb. or 70 p.p.m. of metallic copper calculated on the undried sample. The excess over that required to combine with all the chlorophyll present is probably combined with protein and is readily soluble in gastric juice (Long 1913). Flinn and Inouye (1929) draw attention to the close affinity of copper for proteins and to the protective action of food against copper poisoning. It would seem, however, that such protective action can be effective only in so far as the copper protein is not digested and the copper not converted into a soluble dialysable form.

The most comprehensive investigations on the effect of coppered vegetables on health were those carried out by Taylor, Long and Chittenden for the United States Department of Agriculture in 1913. They found no evidence of toxic action either in man or in animals, but in view of the temporary retention of copper in the liver and its slow excretion they were not in favour of its use for greening vegetables. In this country the question was considered by a Departmental Committee on Preservatives in 1901 and adversely reported upon, although in a minority report it was suggested that greening with copper should be permitted up to a limit of half a grain of metallic copper per pound (70 p.p.m.) and its presence declared. No action was taken on this report. In 1924 another Departmental Committee on Preservatives recommended that the practice of coppering green vegetables should be prohibited, and this was carried into effect by the Preservatives Regulations of 1925.

The Departmental Committee of 1924, in arriving at their conclusion, appear to have laid particular stress on Mallory's results on haemochromatosis (p. 12), results which Mallory himself subsequently admitted to be inapplicable to the amounts of copper in question. It is generally agreed, however, that the Committee were justified on general grounds in condemning the coppering of vegetables. The more recent discovery of the destruction of vitamin C by copper has furnished an additional argument against this practice. A further objection to the presence of copper in canned vegetables is that in unlacquered cans it promotes the corrosion of tin. Spinach greened with copper has been found to contain as much as 0·11 per cent (1,100 p.p.m.) of tin or 8 gr. per lb. (Heuser and Krapohl 1937).

The green colour sometimes shown by olive oil is said to be due to the combination of traces of copper with chlorophyll present in the oil (Marcille 1927).

Zinc has also been used to stabilise the colour of green vegetables and has an effect similar to, but less intense than, copper (p. 116).

In vegetables containing no chlorophyll, copper may cause discoloration. In canned corn, for instance, even as little as 2 p.p.m. may turn the contents of the can black owing to the formation of cupric sulphide (La Que 1935). Even in canned peas, copper may in certain circumstances produce discoloration (Adam and Dickinson 1943).

COPPER HAZE IN WINES. Copper when present in wine to the extent of 0·5 to 4 p.p.m. often causes turbidity, similar to that caused by iron (Ribéreau-Gayon 1930). Copper haze occurs when conditions are such as to favour reduction to the cuprous state, whereas iron haze on the contrary is associated with conditions favouring oxidation to ferric iron (p. 248). In the cask, where a limited amount of air is present, the wine remains clear, since aeration is insufficient to oxidise iron but enough to keep copper from becoming reduced to the cuprous state. When wine containing copper is bottled it gradually becomes turbid owing to reduction of copper and clears again if it is kept uncorked for some time. According to Carles (1918) a red sediment of cuprous oxide is deposited if the turbid wine is centrifuged.

For the removal of copper from wines treatment with alkaline sulphides has been suggested (Ribéreau-Gayon 1935). The slight taste due to this treatment soon disappears. An older device was treatment with tannin and skim milk (Carles 1918).

COPPER IN TOMATOES. In recent years attention has been drawn to the presence of relatively large amounts of copper in tomato purée. This purée is imported in normal times from Italy, Austria, Hungary, Bulgaria, South America, the United States and Canada, and some consignments, notably from Italy, Hungary, and Bulgaria, have been found to contain from 100 to 250 p.p.m. calculated on the dry solids. The greater part of this copper is derived from the vessels in which tomato purée is concentrated.

There is some doubt as to the amount of copper natural to tomatoes, owing partly to uncertainty whether the samples examined may not at some time or other have been sprayed with copper fungicides. Cockburn and Herd (1938) found that out of 26 samples of fresh tomatoes 9 contained over 1 p.p.m. and 2 contained 2 p.p.m. and 2·5 p.p.m. respectively. As the tomatoes had been freed from skins and cores none of this copper was likely to have been due to spraying, and it was regarded by them as natural to the fruit. Needs (1939) found 1·8 p.p.m. in an unsprayed tomato. Shannon and Englis (1940) examined 13 representative samples from Illinois and found from 0·90 to 1·37 p.p.m. of copper.

Figures obtained by me on over 40 samples indicate that unsprayed tomatoes contain from 0·25 to 1·2 p.p.m. of copper. The samples included both home-grown and imported tomatoes, the latter from Holland, Channel Islands, Canaries, West Indies, Cyprus and Morocco. The home-grown ones included several grown by amateurs in different parts of the country and known to be unsprayed. Evidence of spraying could be obtained by washing the tomatoes and testing the washings for copper, or by determining copper in the skin and in the pulp separately, the skin being easily detached after momentary treatment with steam or boiling water. The method of analysis adopted was that given on page 51. In unsprayed tomatoes the copper content of the whole tomato was only slightly more (by 0·1 to 0·2 p.p.m.) than that of the pulp. Copper from spray residue did not appear to penetrate to the pulp, which rarely contained more than 1 p.p.m. and usually about 0·5 p.p.m.

The skin contains, weight for weight, a great deal more copper than the pulp. Horner (1941) found that if copper had accumulated in the soil owing to spraying of successive crops the amount taken up was appreciable. He gives figures between 5 and 10 p.p.m. for copper natural to the skins and derived partly from accumulated spray residue in the soil. Since, however, the weight of the skin is not more than one-fiftieth of the weight of the pulp, this amount of copper in the skin is not more than 0·1 or 0·2 p.p.m. when calculated on the whole tomato.

Tomatoes contain from 5 to 7 per cent of total solids, so that 1 p.p.m. of copper corresponds to 14–20 p.p.m. in the dry solids. Tomato purée as imported is of varying degrees of concentration. The strength most commonly met with is about 25 to 27 per cent of total solids, but a weaker purée of 10 to 15 per cent is also much used. For the purpose of controlling the copper content of tomato purée imported into this country it was suggested that a provisional limit of 20 p.p.m. in the dry solids was adequate to cover any copper naturally present (*Ann. Rept. C.M.O.* 1938). This figure assumes that 1 p.p.m. of copper in the pulp and 5 per cent of total solids are limiting values and that copper due to spraying does not penetrate to the pulp but is removed with the skin before the pulp is processed. If calculated on the higher figures of Cockburn and Herd for copper in pulp referred to above, the suggested limit would have to be raised by a corresponding amount. It was recognised that a requirement of 20 p.p.m. could not be insisted upon for some time, and that for two years (up to January 1940) imports of tomato purée containing up to 50 p.p.m. in the dry solids should be permitted. Later on it was decided that copper up to 100 p.p.m. should be allowed until January 1940 and that a limit of 50 p.p.m. would have to be complied with after that time. On the outbreak of war the operation of the 50 p.p.m. limit was postponed, and it has since remained at 100 p.p.m. (*Analyst*, 1940, **65**, 220). It should be noted that this limit has no statutory force. It represents a line of action which medical officers of health at the ports have agreed to take under the general powers conferred upon them in respect of imported food, and it is open to an importer to contest this action in a court of law in respect of any particular consignment.

The effect of copper in tomato purée upon the vitamin C content does not seem to have received much attention. Since evaporation is carried out under reduced pressure in absence of air the loss of vitamin C due to oxidation by copper may not be so great as with greened vegetables which used to be treated with copper sulphate in open vessels before canning. Moreover, the amount of copper present in most tomato purées is much less than that formerly added to green vegetables.

In order to arrive at the amount of copper in the dry solids of tomato purée it is necessary to determine not only copper but also total solids. In evaporating tomato juice or pulp to dryness in a drying oven in the ordinary way there is appreciable decomposition of sugars, etc., and consequent loss of solids. Trustworthy results can be obtained by evaporating to dryness under reduced pressure at a temperature not above 70°, preferably in a vacuum oven, or failing this, in a weighed round-bottomed flask of 50 to 100 ml. capacity connected to a good water pump and immersed in a water bath at 70° (Analytical Methods Committee 1941).

COPPER IN VARIOUS FOODS. The following is a rough division of some common foods according to the amount of copper, either natural or adventitious, recorded as having been found in them.

Nil to 0·5 p.p.m.—milk, butter, cheese, refined sugar, honey, margarine, lard, suet.

0·5 to 2 p.p.m.—fresh fruits and most vegetables, wheat flour, cornflour, bread, macaroni, pearl barley, tapioca, biscuits, dried milk, fish, meat, eggs, beer and stout, vinegar, cider.

2 to 10 p.p.m.—wholemeal flour and oatmeal and preparations of these, peas and beans, dried fruits, table salt, mustard, sheep's kidneys, bacon, mushrooms, British wines, raw beet sugar.

10 p.p.m. and over (p.p.m. in figures)—tea, coffee, cocoa and chocolate (10 to 30), cocoa shell (200), coffee and chicory essence (20), nuts (10), pepper (10), currants (17), curry powder (10), gravy powder (17), liver (50), lambs' and calves' liver (100), crustaceans and shell-fish, especially oysters (up to 400), gelatin (25), tomato purée (10 to 50), yeast (50 to 100), yeast extract (20 to 60).

The above figures refer to copper in the foods as sold. If the copper content is expressed as parts per million of dry matter present, the amounts may of course be very much higher.

Many results for copper in various foods have been tabulated by McCance and Widdowson (1940), and similar results have been obtained by me in a series of analyses carried out by the method given on page 51. From these figures combined with a quantitative record of the food, comprising 63 different items, consumed over a period of four weeks in a household of two adults on a weekly income of about £5, the daily intake of copper per head was estimated to be 1·7 mg. No doubt the intake will vary considerably according to locality and diet and will be higher where copper pipes are used for domestic water supplies. Tompsett's estimate of the average copper intake is 2 to 2·5 mg. daily (1934). Orr's figures for a number of Scottish families of the poorer class range from 1·5 to 2·5 mg., and Leverton and Binkley (1944) give 2·65 mg.

In many foods, as for instance liver, crustaceans and shellfish, tea, coffee, cocoa, nuts and mushrooms, a high copper content appears to be a natural characteristic of the food. Sometimes, as in tea, pepper and currants, it is largely due to the material having been dried down to a fraction of its original weight, so that all non-volatile constituents become concentrated. None of the copper in tea or coffee finds its way into the infusion. In some other foods high in copper it has obviously been introduced during processes of manufacture in copper vessels.

The copper in oysters ranges from a trace up to several hundred parts per million and is due to contamination of estuaries by industrial and mining effluents. Dutch oysters stated not to have been in contact with copper at any stage have shown 10 to 30 p.p.m. Apparently oysters readily extract and accumulate traces of copper from water (p. 13).

Seventy or eighty years ago it was customary, according to Hassall (1876), to add copper sulphate to flour as an improver. The amount used seems to have been equivalent roughly to 5 or 6 p.p.m. of copper in the bread, but sometimes much larger quantities were met with. No instances of this have been reported for many years. The action of copper sulphate is no doubt that of an oxidation catalyst (cf. bromates, p. 337). Another practice referred to by Hassall, which appears to be obsolete, is that of painting a solution of copper sulphate on the rind of cheese to prevent attack by insects.

A possible source of copper in food is the bronzing powder sometimes used for colouring paper in which confectionery and chocolate are wrapped. This powder consists mainly of copper and zinc and often comes off if the paper is rubbed. The so-called 'nonpareils' or small gold beads used in confectionery are coated with brass foil: beads of the size of large pinheads or small shot contain roughly 10 per cent of copper and 3 per cent of zinc.

Domestic water supplies frequently contain copper owing to the employment of copper pipes in place of lead where the water is plumbosolvent (p. 74).

COPPER AND PUBLIC HEALTH. In former days copper in food seems to have been regarded as a major menace to health on a par with arsenic and lead (Hassall 1876). This was due to the well-known irritant and inflammatory action on the stomach of copper salts in large doses, and to the excessive amounts often used in colouring confectionery, pickles, etc. The whole question of copper in food was reviewed by Tschirch in 1893, who concluded that there was no evidence of chronic copper poisoning in man and that the copper which is normally present in food, or which may be introduced by the carefully controlled greening of vegetables, is without adverse effect on health. Taylor, Long and Chittenden's experiments for the United States Department of Agriculture in 1913, which led to no conclusive results, have already been referred to (p. 25). In this country the use of artificial colouring matters containing copper, which includes the greening of vegetables with copper salts, is illegal (p. 26), and a limit has been prescribed for edible gelatin: there are no other statutory standards or limits for copper in foods, except the general provision of Section 1 of the Food and Drugs Act, 1938, which prohibits the addition to food of any injurious substance. In several other countries the addition of copper to vegetables is permitted up to a limit of 100 p.p.m.

In discussing the public health aspect of toxic metals and other foreign substances in food there are two main questions to be considered; (i) the purely scientific question to what extent and in what quantities they are injurious, and (ii) the purely administrative question whether the amount allowable in foods should be regulated by statute or whether, as at present, local authorities should be free to take action if they think fit, with ultimate reference to courts of law for decision according to the evidence available. In this country the only statutory schedules in force, with the exception of two recent enactments, relate to preservatives and colouring matters.

On the scientific question, there is for most foreign substances in food no sharp dividing line between what is clearly toxic and what is undoubtedly harmless. Between these two stages lies a wide range of intake where it is extremely difficult to decide whether or not there is any remote toxic action. With improved methods of animal experiment, involving careful observation of growth rates and fertility in several different species on strictly controlled diets, and minute post-mortem examination of tissues, this gap can be narrowed considerably. It may, however, be contended that differences in digestion, assimilation and metabolism between man and animals are usually so great, and the recognition of slight digestive and nervous disorders in animals so uncertain, that such experiments can hardly ever be conclusive. Until it can be shown conclusively that a given quantity of a particular foreign substance is absolutely harmless and devoid of any remote toxic effect it should, as far as is humanly possible, be kept out of food. Can we safely fix limits of intake for certain poisons below which no possible toxic action need be apprehended, or must such substances, when repeatedly ingested in quantities which are apparently non-toxic, be assumed to have some adverse effect, however small, on metabolism and to impose an extra strain at some point in the mechanism of the body or the processes of elimination ? It is a perfectly sound contention that the one who consumes the food, and who is the chief sufferer if there is anything wrong with it, is entitled to the fullest possible protection in respect of its purity. On the other hand, it is clear that action on these lines, if pushed to its logical conclusion, must to some extent hamper research in food manufacture and veto many processes which may in fact be to the advantage of the consumer. It can be urged that where exhaustive animal experiment has shown no sign of injury of any kind, there is no reason to fear any adverse effect on man and no justification for imposing limits and prohibitions which may harass traders and impede progress without conferring any apparent benefit on the consumer. It may perhaps be argued that many organs and tissues of the body—liver, kidneys, digestive tract—have as one of their functions the removal of unwanted or toxic substances in food, and it is hard to believe that they do not perform this duty efficiently. To this it may be replied that it is not only the normal healthy individual who must be considered, but also the invalid or person of delicate constitution who may be particularly susceptible and whose organs of elimination may not be so efficient.

Thus there are always, on the one hand, those who demand experimental proof of injury to health before any action is taken and, on the other, those who would exclude from food anything which may reasonably be regarded with suspicion. Agreement is not likely to be reached without far more complete knowledge of human physiology than we possess at present. Meanwhile, any preference must be on the side of the consumer. We are bound to take into consideration the chances of remote action beyond the reach of experimental proof and to insist upon a wide margin of safety.

In favour of the case for statutory standards or limits for toxic elements in foods, it may be urged that without such standards uniformity of action throughout the country is impossible, whether by analysts in reporting upon samples, by local authorities in initiating proceedings, or by courts of law in deciding how much of a toxic element is allowable and how much is excessive. For the consuming public uniformity in these matters is not necessarily an advantage, but for the trader the difficulties and uncertainties of a system which makes it possible for different criteria to be applied in different circumstances and in different parts of the country, involving him possibly in unforeseen litigation, are obvious. For the local authority also, a prosecution undertaken without the support of a statutory standard is likely to be risky and expensive.

One of the objections to legal standards is that where foods are subject to wide variations in quality, standards must be lenient so as not to prevent the sale of foods which may be wholesome, although of lower quality. This also applies to some extent when two or more standards are made for different grades of a particular food. Thus standards may often be of little practical value as a protection to the consumer except to exclude the very lowest qualities ; in fact, they may sometimes be to his disadvantage in that the tendency is for producers to work to the standard and to debase the higher grades of food. This is seen, for instance, in the percentage of water in butter, which used to be from 11 to 12 per cent in the better qualities, but which is now regularly brought as near as possible to the statutory standard of 16 per cent. A standard for toxic metal content must to some extent be open to the criticism that when it has been complied with there is no further incentive to improvement. Again, a statutory standard may, in some particular application, prove to be too stringent. However carefully a schedule is prepared, it is seldom possible to take every factor into account or to make adequate allowance for changing circumstances. Legal standards when once made are difficult to alter and may remain on the statute book long after scientific research and industrial progress have modified, or even radically altered, the basis upon which they were made. The ideal system is one which enables ready adjustment to be made when unforeseen contingencies arise, and which acts on the principle that with food, more than with any other commodity, the interest of the consuming public must always come before commercial advantage and administrative convenience.

Statutory standards for food in general are discussed in the report of the Departmental Committee on the Composition and Description of Food (1934), and a number of such standards have lately been made by orders under the Defence (Sale of Food) Regulations, 1943. So far, however, these orders have dealt chiefly with the composition of particular foods or classes of food for the purpose of price control, and in only a few instances has action been taken in respect of standards or limits for toxic elements.

Whatever the future policy on these questions, for the present those concerned with the day-to-day administration of the Food and Drugs Act must form some opinion as to what amounts of any particular toxic element are reasonable and what are excessive, and must interpret, as best they can, the meaning of the word 'injurious' in Section 1 of that Act. Copper, apart from its irritant and obviously toxic action in large doses, is perhaps the most active of all metals as an oxidation catalyst and is particularly destructive of vitamin C. It strongly inhibits bacterial growth and may conceivably affect bacterial action in the intestine, action which may be of significance in digestion and metabolism. Furthermore, there are grounds for believing that the absorption of copper into, and its excretion from, the liver may be considerable. For these reasons its presence in food should be controlled, and the use of unprotected copper vessels in food manufacture should be discouraged. On the other hand, in the absence of any reasonable suspicion that chronic poisoning may result from the repeated ingestion of very small quantities, the restrictive measures appropriate to lead and arsenic are not required. It would seem that if the grosser and more obvious cases of contamination are dealt with, particular attention being paid to liquids, the consumer will be adequately protected.

Foods in which excessive amounts of copper are often met with include fresh and preserved fruits and vegetables, jam, spices, beverages and gelatin. In fruits and vegetables some allowance must be made for copper introduced by spraying with copper fungicides, a long-established practice to which objection can hardly now be taken, provided that it is not overdone. The natural copper content of most fresh fruits and vegetables is rarely more than 2 p.p.m., and an extra 3 p.p.m. would be a liberal allowance for spraying, making 5 p.p.m. in all. In dried produce the amount of copper would, of course, be much higher, owing to concentration in drying.

The water content of most fresh fruits and vegetables lies between 80 and 95 per cent, so that the above figures would correspond to 10–100 p.p.m. in the dry solids according to the nature of the sample. Most fruits and vegetables contain much less than 20 p.p.m. of copper in the dry solids, exclusive of spray residue, and where spray residue is absent or can be eliminated in the process of manufacture this amount will only rarely be exceeded (e.g. tomatoes, p. 27). Beer, stout, mineral waters, cider and vinegar should not contain more than 3 p.p.m., and even this is mostly due to brass and copper plant and fittings. The copper in beer immediately after fermentation is seldom more than 0·25 p.p.m. owing to its removal by yeast (Gray and Stone 1942).

For domestic water supplies Thresh (1925) suggests a limit of 1·4 p.p.m. Schneider (1933) would allow up to 5 p.p.m., but this would seem too high in view of the probability that copper is more irritating to the stomach and more readily absorbed when ingested in liquids, particularly water, than in solids. On the other hand, the United States Public Health Service limit of 0·2 p.p.m. seems unduly stringent (Wilkinson and Wilson 1941). In most liquids the presence of copper in any considerable amount can be detected by taste. 2 p.p.m. will give a metallic taste to drinking-water (Spitta 1932). Some cider experts claim to be able to tell by taste when only 0·8 p.p.m. is present, but the ordinary palate would hardly detect less than 3 or 4 p.p.m.

Imported wines, and British wines made from imported must and raisins (Hancock 1924), frequently contain more than 5 p.p.m. owing partly to treatment of the grapes with copper fungicides but mainly to the use of copper plant and appliances in bottling. In Switzerland the limit for wine is 10 p.p.m.

In meat essences, concentrated soups, etc., a high copper content may be due to the use of a low grade of gelatin made from hide waste. Gelatin can be produced free from copper, and this may be of some importance when it is required for bacteriological work owing to the sensitiveness of some bacteria to copper. The same applies to commercial peptones. Under the Edible Gelatin (Control) Order, 1947, a limit of 30 parts of copper per million has been prescribed for this product. The United States limit is also 30 p.p.m.

Cereal grains contain more natural copper than fruits and vegetables. This is nearly all in the outer seed coats and is therefore highest in wholemeal products. A limit of 10 or perhaps 15 p.p.m. should be sufficient to cover the copper naturally occurring in cereals.

Foods such as liver, crustaceans, oysters and other shellfish, which contain naturally a high and variable amount of copper, are in a class by themselves, and the recognition of extraneous copper may not be possible without knowledge of the conditions under which they have been produced or prepared.

THE DETERMINATION OF COPPER IN FOOD

Analytical methods for the determination of copper in food can be roughly divided into three main groups, according to the means adopted for eliminating interference by iron and other metals and by calcium phosphate, etc. :

(i) Separation of copper by electrolysis.
(ii) Separation of copper by precipitation as sulphide.
(iii) Colorimetric determination after removal of iron as hydroxide or after de-ionising it with ammonium citrate or pyrophosphate.

These methods must usually be preceded by destruction of organic matter, although from certain foods (wines, mineral waters, etc.) copper may sometimes be separated without previous ashing.

DESTRUCTION OF ORGANIC MATTER. Most materials can be ashed direct in a muffle furnace at a temperature not higher than 500° without loss of copper. It has been found that old silica dishes, the surfaces of which have been attacked by ash constituents, are apt to absorb copper and the absorbed copper is difficult to recover. If the ash is sulphated and new dishes are used there is no loss of copper. Comrie (1935) adds magnesium nitrate to the sample to keep the ash bulky and out of intimate contact with the surface of the dish. High (1947) uses nitric acid to ensure complete extraction of copper. Similarly, in the ash of materials rich in silica, copper may be in a state practically insoluble in acids unless the silica is removed by heating with hydrofluoric acid. In dry ashing, organic matter must be completely destroyed ;

if ashing is incomplete the amount of copper found may be too low. Porcelain dishes cannot be used for ashing foods in which copper is to be determined since the glaze may contain copper and contaminate the ash. Elvehjem and Lindow (1929) found as much as 0·33 mg. of copper in the glaze of a 3-inch porcelain dish. It has been suggested that since copper acetate and formate may yield traces of a volatile copper compound on ignition, there may be a loss of copper from this cause in dry ashing.

Sylvester and Lampitt (1935) found that when using a sulphuric acid wet combustion method for milk the results were always slightly lower than those obtained by dry ashing in silica dishes. They attributed this to the adsorption and retention of copper by small amounts of calcium sulphate. When calcium sulphate was brought into solution by heating with potassium carbonate as in the method of Roche Lynch, Slater and Osler (p. 88) the results agreed perfectly with those obtained by ashing in silica dishes. In most instances it is possible to bring this adsorbed copper into solution by filtering off the calcium sulphate and heating it with diluted (1 : 1) hydrochloric acid.

The destruction of organic matter is discussed further in connection with the determination of lead (p. 84).

ELECTROLYSIS. With some foods copper may be separated in a pure state by direct electrolysis of a solution of the ash in dilute sulphuric acid. The sulphated ash is dissolved in about 50 ml. of dilute (N/2) sulphuric acid containing a few drops of nitric acid, which has the effect of making the deposit more coherent. A platinum dish may be used as cathode and a coil of platinum wire as anode, but it is better to use a cathode of platinum gauze with a large surface in proportion to its weight. If more than a trace of nitric acid is present an anode of platiniridium is preferable as it is more resistant than platinum. With a current of 0·3 ampere and a current density of 0·01 amp. per cm.^{2}, 10 mg. of copper is completely deposited in 1½ hours (Lampitt and others 1926). When the electrolysis is finished the solution must be replaced with distilled water without interrupting the current, or copper may be redissolved. The copper deposit is finally washed with alcohol and ether, or with acetone, dried and weighed.

If much nitric acid is present, nitrous acid may be formed at the cathode, and this interferes with the deposition of copper. It may be removed by adding urea. Frear (1939) determines copper in spray residues on fruit by electrolysing a solution of the ash in 100 ml. of dilute nitric acid containing about 6 g. of ammonium nitrate and 1 g. of urea. The copper is deposited on a platinum gauze cathode, the anode being of platinum wire and the current not more than 0·5 ampere. In the German official method for coppered vegetables (*Gesetze und Verordnungen*, 1928, **20**, 13) the ash of the sample is dissolved in 10 ml. of 25 per cent nitric acid and the solution made up to 100 ml. with water. The electrolysis is thus carried out in 2·5 per cent nitric acid.

Springer (1929) determines copper in coppered vegetables by direct electrolysis without previous destruction of organic matter. 100 to 150 g. of the material are well pulped and heated on a steam bath with about 10 ml. of concentrated nitric acid. The mixture is made weakly alkaline with ammonia, acidified with 10 ml. of sulphuric acid (1 : 1), made up to about 300 ml. with water and electrolysed at 80° to 90° with a current of 4 to 5 amperes. A rotating platinum anode combined with a stirrer and a stationary platinum-gauze cathode are used. The separation of copper is complete in 10 to 20 minutes. The electrodes are washed and adhering vegetable fibre removed without interrupting the current, and the copper dissolved from the cathode with 10 ml. of concentrated nitric acid. The nitric acid solution is made slightly alkaline with ammonia, 10 ml. of sulphuric acid (1 : 1) added and the solution made up to about 150 ml. It is again electrolysed at 80° to 90° with a current of 4 to 5 amperes for 20 minutes, after which the cathode is washed, dried with alcohol and ether and weighed.

Obviously, unless the food contains a great deal of copper a large sample is required in order to get a weighable deposit; further, the sample must be free from tin, arsenic, antimony, bismuth and lead, all of which may contaminate the copper. Tin especially is liable to be deposited with copper, and as many foods contain small amounts of tin, electrolysis is not in general trustworthy unless copper is first separated by chemical methods or unless the copper on the cathode is dissolved off and re-electrolysed or determined in some other way. If tin is the only other metal present likely to interfere it may be made inert by conversion into insoluble metastannic acid with nitric acid.

A number of micro-electrolytic methods have been described based on that of Pregl (1930) in which copper is deposited on a small platinum-gauze cathode from about 5 to 10 ml. of solution, using currents varying from 0·007 to 0·5 ampere (Keilholz 1921, Fontès and Thivolle 1923, Maquenne and Demoussy 1919, Debordes 1929, Lindsey and Sand 1935, Lindsey 1938). In some of these the copper deposit is weighed on a micro-balance, but usually it is dissolved from the cathode and determined colorimetrically.

PRECIPITATION AS SULPHIDE. Normally the strength of acid from which copper is precipitated as sulphide by hydrogen sulphide should not exceed 2N, but Harry (1931) precipitates from 15 per cent hydrochloric acid, and Ansbacher and others (1931) from a solution containing 15 per cent by volume of sulphuric acid. The acid concentration should not be less than 0·3N (pH 0·4) to prevent precipitation of traces of metals of the iron group. According to Fairhall and Richardson (1930) copper and zinc are precipitated quantitatively between pH 2·5 and 4·0 as sulphides uncontaminated with iron, but it would seem doubtful whether at this low acidity complete separation from iron can be effected. The Analytical Methods Committee of the Society of Public Analysts, in giving methods for the determination of lead and other poisonous metals in food colours (p. 94), recommended that lead and copper should be precipitated together by hydrogen sulphide at about pH 4·0, at which degree of acidity only a very small amount of iron comes down, this being eliminated at a later stage by addition of ammonium citrate.

When the amount of copper is very small it is more readily precipitated in presence of mercuric sulphide, mercury being afterwards removed by calcining the mixed sulphides. Copper sulphide may also be precipitated with sodium thiosulphate in sulphuric acid solution ; free sulphur is formed and serves to adsorb and carry down cupric sulphide. The cupric sulphide may be dissolved from the filter with nitric acid or by oxidation to sulphate with a mixture of one volume of bromine water and two volumes of 10 per cent sulphuric acid solution (McFarlane 1932), bromine being afterwards driven off by heating. Copper is then determined by one of the several colorimetric methods available.

ELIMINATION OF IRON BY OTHER METHODS. Iron interferes with many of the colorimetric methods of determining copper. It can be removed by precipitation with ammonia or kept in solution and its effect neutralised by de-ionisation with alkaline citrate or pyrophosphate.

Precipitation with Ammonia. According to Callan and Henderson (1929) iron can be completely removed without loss of copper by adding excess of ammonia to the solution and filtering off ferric hydroxide, copper being determined in the filtrate. Other workers (Toporescu 1920, Haddock and Evers 1932, Hamence 1934) have found that a single precipitation does not separate the two metals completely and that copper is obstinately retained by ferric hydroxide whatever the conditions of precipitation. Lampitt and others (1926) carry out a double precipitation, the ferric hydroxide first obtained being filtered off, re-dissolved and re-precipitated with ammonia. The combined filtrate contains the whole of the copper. This procedure is followed also for copper in food colours by the Analytical Methods Committee of the Society of Public Analysts (1939), but is somewhat laborious, particularly with such foods as milk powders (cf. also Cockburn and Herd 1938).

De-ionisation of Iron with Pyrophosphate. Warburg (1927) used sodium pyrophosphate to neutralise the effect of iron in his method of determining copper by the catalytic oxidation of cysteine to cystine. It was also used by

McFarlane (1932), Tompsett (1934) and Hoar (1937) in the determination of copper with sodium diethyl-dithiocarbamate (p. 49) and by Elvehjem and Hart (1931) in their modification of the Biazzo pyridine method (p. 44).

The iron in ferric pyrophosphate is un-ionised and does not react with thiocyanate or diethyl-dithiocarbamate. Eden and Green (1940) point out that when ammonium pyrophosphate is used the completely de-ionised compound is an iron ammonium pyrophosphate, and for the immobilisation of iron to be fully effective the solution must contain plenty of ammonium salts and a large excess of pyrophosphate. This treatment does not affect the reactivity of copper, although pyrophosphates in neutral or acid solution inhibit the oxidation of vitamin C by copper (p. 22).

De-ionisation with Alkaline Citrate. Alkaline pyrophosphate, while it eliminates interference by iron, does not prevent the precipitation of calcium phosphate, which is apt to prove troublesome in the examination of milk and milk products. Ammonium citrate not only de-ionises iron but also prevents the precipitation of calcium phosphate. Haddock and Evers (1932) in applying this to the diethyl-dithiocarbamate method found that 2 g. of citric acid were sufficient for quantities of iron up to 100 mg. Ferrous iron should be absent.

The action of ammonium citrate and pyrophosphate in de-ionising iron has been thoroughly discussed by Eden and Green (1940). Iron in ferric ammonium citrate is completely de-ionised in presence of sufficient ammonium and citrate ions. Manganese is also de-ionised both by citrate and pyrophosphate. In presence of phosphates of magnesium and aluminium, alkaline citrate does not prevent formation of a precipitate, and separation of copper as sulphide may be necessary.

The remarks on special methods for calcium phosphate in connection with lead on page 88 also apply to copper, and Roche Lynch's method is equally applicable to the determination of copper.

Iron may also be separated from copper in micro-analytical work by saturating the solution with salt and extracting it with ether containing 5 per cent of formic acid (Ramsay 1944).

XANTHATE COLORIMETRIC METHOD. This method is based on the formation of a yellow colour when a solution of potassium xanthate, $CS(OC_2H_5)SK$, is added to a solution containing a small amount of copper. It can be used for quantities as low as 0·001 mg. in 100 ml., but it has the disadvantage that the solution must be absolutely neutral since opalescence occurs on either side of neutrality. Slight opalescence cannot sometimes be avoided. Moreover, yellow colours are difficult to match exactly with standards.

Details of the method are given in the A.O.A.C. handbook (1940). According to Hess, Supplee and Bellis (1923) it is trustworthy for 0·05 p.p.m. of copper in milk. Nickel gives a colour similar to that given by copper and, if present, must be removed (King and Etzel 1927).

FERROCYANIDE METHOD. This method is suitable for classification of samples but is not very accurate (Lampitt and others 1926). Its range is from 0·07 to 0·40 mg. of copper. The sulphated ash of the sample is freed from iron by double precipitation with ammonia, the ammonia removed by boiling and ammonium acetate added. Copper is then determined colorimetrically with potassium ferrocyanide. The object of adding ammonium acetate is to keep the pH of the solution as near as possible to 7·0. If the solution is too acid the colour is yellow, and if alkaline the red colour of cupric ferrocyanide may not develop. The method has been tentatively adopted in the United States for the determination of copper in gelatin (*J. Assoc. Off. Agric. Chem.*, 1939, **22**, 84).

PYRIDINE THIOCYANATE (BIAZZO) METHOD. The Biazzo method originated in an observation by Spacu (1925) that copper can be separated from mercury by adding pyridine and ammonium thiocyanate to the boiling solution. In presence of copper, pyridine gives a dark blue colour and on adding solid ammonium thiocyanate a green flocculent precipitate of copper dipyridine dithiocyanate, $Cu(C_5H_5N)_2(SCN)_2$, is produced: mercuric salts give no precipitate.

Biazzo (1926) found that the green copper compound could be extracted with chloroform and made this the basis of a method of determination. Five grammes of the sample are ashed, the residue treated with a few drops of 50 per cent hydrochloric acid, the solution evaporated to dryness and the residue taken up with 20 ml. of water and transferred to a separator. Sodium hydroxide solution is added until the liquid is just alkaline, then acetic acid until it is just acid, followed by a few drops of concentrated solutions of pyridine and potassium thiocyanate. After extraction with chloroform the green colour of the chloroform extract is compared with that of standards.[1]

Elvehjem and Lindow (1929) made some minor modifications in the method and found it very satisfactory for amounts of copper over 0·02 mg. Iron, if present, produces a brownish (ferric) or greenish (ferrous) coloration in the chloroform, and with milk or milk products calcium phosphate is precipitated and interferes with complete extraction of copper, unless the copper has previously been separated as sulphide. Chalk (1930) finds that if tartaric acid is substituted for acetic acid iron does not interfere provided that it has been oxidised to the ferric state, and calcium phosphate is not precipitated. Tartaric acid thus behaves like the citric acid used in other methods. Details of the method and of the modifications necessary when much calcium phosphate is present are given in Chalk's paper.

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[1] Colorimetric determinations may be carried out in several ways, subjectively by visual comparison or objectively by measuring the effect of the transmitted light on a photographic plate or a photoelectric cell. A number of devices are used for varying the nature and amount of the incident light so as to obtain measurements of optical density at the most favourable spectral range and light intensity for the coloured solution under examination. A wide choice of photometers, photoelectric absorptiometers, and spectrophotometers is available for this purpose. In all these instruments the comparison is made in the first instance with a standard solution, and if the coloured solution does not obey the Beer-Lambert law (i.e. if the ratio between absorbed and transmitted light is not constant for successive equal increments of concentration or thickness of solution) a calibration curve must first be constructed for the solution in question. There is no doubt that objective methods, such as those depending on the use of a selenium cell, give more precise results than can be obtained by the older methods of visual comparison with standards, and that for routine work they are quicker and more convenient, but for occasional determinations they are to be preferred only if the apparatus is ready to hand and has been suitably calibrated by means of one or more standard solutions. In the following pages the term ‘ comparison with standards ’ is to be understood as including all methods of colour measurement which depend, in the first instance, upon such calibration.

Nickel and cobalt give blue and pink pyridine thiocyanates respectively and must therefore be absent. Silver and mercurous salts give insoluble thiocyanates which interfere with the extraction of copper by chloroform. Cyanides must be absent as they prevent the formation of copper pyridine thiocyanate. Ansbacher and others (1931) use bromobenzene for the extraction in place of chloroform.

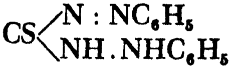
PHOSPHOMOLYBDIC ACID TITRATION METHOD. This method, due to Fontès and Thivolle (1923), depends on the fact that phosphomolybdic acid dissolves finely divided metallic copper and is thereby reduced, with production of a blue solution probably containing the complex $(Mo_2O_5,4MoO_3)_2H_3PO_4$ (cf. p. 198). On titration with permanganate this complex is reoxidised to phosphomolybdic acid. Copper in solution is reduced to metal either by electrolysis or, in absence of iron, by precipitation with α-nitroso-β-naphthol and ignition in hydrogen, and the metallic copper is dissolved in a few millilitres of phosphomolybdic acid solution. Further details of the method are given in the *Analyst*. Guillemet (1932) states that this method is suitable for amounts of copper down to 0·01 mg., and Debordes (1929) uses it for copper in wines after separating the copper first as sulphide and then electrolytically.

QUINOSOL METHOD. Quinosol, stated to be a double sulphate of potassium and 8-hydroxyquinoline, is a well-known antiseptic. It gives a precipitate with solutions containing traces of copper (Saul and Crawford 1918). Lampitt, Hughes, Bilham and Fuller (1926) introduced a gravimetric method of determining copper in foods based on this reaction. After wet oxidation of the sample the sulphuric acid was fumed off, and the solution diluted to 50 ml. and made slightly alkaline with ammonia. The copper compound was obtained by double precipitation with quinosol, filtered off, dried and weighed. It contained 18·55 per cent of copper, and Lampitt and his co-workers gave it the formula $C_9H_7N.OCu.SO_4K$. They pointed out that manganese also forms a precipitate, and if this metal is present copper must afterwards be separated from it by electrolysis. Fuller details of the method are given in their paper.

Berg in 1927 introduced 8-hydroxyquinoline or 'oxine' as an analytical reagent for the determination of a number of metals, which he grouped according to the acidity or alkalinity of the solutions from which they are precipitated and the presence or absence of hydroxides or tartrates of sodium and ammonium (cf. under aluminium). Berg assigned the formula $(C_9H_6ON)_2Cu$ to the precipitate given by oxine with copper salts in alkaline sodium tartrate solution. This compound should contain 18·0 per cent of copper as against 18·4 per cent calculated from the formula given by Lampitt for the double sulphate. It is not clear whether these two compounds may not be in fact identical. Berg titrates the oxine precipitate with standard bromide-bromate solution (cf. p. 415). Zinc is also precipitated by hydroxyquinoline in alkaline solution and copper must therefore first be separated from it.

IODIMETRIC METHOD. This is the well-known method of de Haen and has been adopted as a tentative method for copper in amounts over 1 mg. by the Association of Official Agricultural Chemists (1945). Iron must be absent since ferric iron liberates iodine from potassium iodide, and copper is therefore first separated either as sulphide, by boiling the solution with sodium thiosulphate, or electrolytically. Lasausse (1923) separates copper by precipitation on aluminium and dissolves it in nitric acid. An alternative and simpler method is to de-ionise iron with pyrophosphate and carry out the titration in acetic acid solution (Ley 1917).

For small amounts of copper the method has the disadvantage that the end-point of titration with N/100 sodium thiosulphate is not sharp. The method does not work well in presence of tin salts (Lampitt 1926).

DITHIZONE METHOD. Copper combines with diphenyl-thiocarbazone ('dithizone')  $\mathrm{CS}\left\langle\begin{matrix}\mathrm{N:NC_6H_5}\\ \mathrm{NH.NHC_6H_5}\end{matrix}\right.$ (cf. pp. 86, 124), forming a brown compound soluble in chloroform and carbon tetrachloride. When a solution of dithizone in chloroform, which is deep green in colour, is shaken with an acid, neutral or weakly ammoniacal solution of a copper salt the whole of the copper passes into the chloroform turning it brown. If the solution is strongly acid (2 to 3 per cent of sulphuric acid) the only other metals extracted by dithizone are mercury and silver. If the acid solution contains hydriodic acid or potassium iodide only copper is extracted. At high degrees of acidity, however, the extraction of copper is not so readily effected as in slightly acid solutions and requires violent shaking for at least a minute with three successive portions of the chloroform-dithizone solution. Stolze (1936) extracts from 2 per cent sulphuric acid and in Sylvester and Lampitt's method (1935) the solution has an acidity of N/7 hydrochloric acid or about pH 0·8. Wichmann (1939) recommends an acidity corresponding to pH 4·0 to 5·0, at which, he says, copper is rapidly and completely extracted by dithizone, but at this slight degree of acidity zinc, mercury, silver, bismuth and cadmium are extracted as well as copper (Sylvester and Hughes, p. 124). Zinc, bismuth and cadmium can be removed, and silver precipitated, from the dithizone extract by washing it with dilute hydrochloric acid. Mercury can be removed by washing with potassium iodide solution (Bendix and Grabenstetter 1943, cf. p. 464). The usual strength of the chloroform solution of dithizone used for these extractions is 0·1 per cent.

Extraction with dithizone by any of the above methods is an admirable way of separating copper from other metals before its determination with diethyl-dithiocarbamate, but in order to use the dithizone extract itself for the colorimetric determination of copper certain precautions must be taken. The chloroform solution of dithizone must be carefully purified by extracting it with ammonia, discarding the chloroform layer, re-precipitating the dithizone with dilute hydrochloric acid and re-dissolving it in chloroform. This removes the yellow oxidation products which are always present in commercial dithizone and which being soluble in chloroform would affect the final colour. Ferric iron must be absent, owing to its oxidising action. It may be reduced with bisulphite (Stolze 1936) or de-ionised with pyrophosphate or citrate. After extraction of copper excess of dithizone is removed from the chloroform solution by shaking with dilute ammonia and the colour of the copper-dithizone compound remaining in the chloroform compared with standards containing known amounts of copper. The method is stated to be sensitive to 0·001 mg. of copper.

The extraction of metals with dithizone and the various ways in which it may be used for colorimetric work are further discussed below in connection with lead (pp. 85, 91) and zinc (p. 124). A tabular summary showing the conditions under which different metals react with dithizone is given on page 131.

DIETHYL-DITHIOCARBAMATE METHOD. Sodium diethyl-dithiocarbamate, $(C_2H_5)_2N.CS.SNa$, gives with solutions containing copper a brown precipitate of the normal salt, soluble in amyl alcohol and in ether. According to Callan and Henderson (1929) this reagent will detect one part of copper in 100 million parts of water. The colour of the precipitate is the same in acid, neutral and alkaline solution, but it is not formed in presence of cyanide.

The colour, being due to a colloidal precipitate, is sometimes difficult to match with standards. By extraction with amyl alcohol or other organic solvents a clear solution is obtained. Amyl alcohol extraction increases the range as well as the sensitivity of the method, since the copper compound is soluble and there is not the same tendency to precipitation that there is in aqueous solution. According to Thatcher (1933) *iso*-amyl alcohol, being less soluble than amyl alcohol, gives a sharper separation. Amyl or *iso*-amyl alcohol extraction has also the advantage that it separates the copper compound from the similar yellow compound given by iron. The Analytical Methods Committee of the Society of Public

Analysts (1939) prefers not to extract with amyl alcohol and finds that the addition of gum arabic gives a bright golden-brown colour suitable for colorimetric comparison and stable for several hours. Iron is either removed by precipitation with ammonia or de-ionised with ammonium pyrophosphate or citrate, the last named having the advantage of keeping calcium phosphate in solution.

Lead gives a turbidity with diethyl-dithiocarbamate and zinc also, if in large amount, may interfere. Both of these, if present, can be removed by ferric hydroxide precipitation or the zinc turbidity may be prevented by adding ammonia. Bismuth in large amounts gives a yellowish-green precipitate extracted by amyl alcohol and is not de-ionised by ammonium citrate. Coulson (1937) found that copper could still be determined in presence of bismuth by using a blue filter when matching the colours. The colour due to bismuth can be distinguished from that given by copper by the fact that it is not destroyed by cyanide. Manganese gives a white turbidity with diethyl-dithiocarbamate which dissolves in amyl alcohol giving a pink solution, but like iron is de-ionised by alkaline pyrophosphate.

Copper in biological material can be completely extracted with dilute trichloracetic acid which also has the advantage of precipitating proteins. Tompsett (1935) grinds the material with a 10 per cent solution of trichloracetic acid and broken glass, and determines copper in the filtrate with diethyl-dithiocarbamate after de-ionising iron with sodium pyrophosphate or citrate or both.

Eden and Green (1940) have given a quick method for small quantities of material. One to five grammes of the sample according to its moisture content are oxidised with sulphuric, perchloric and nitric acids in an 8″ × 1″ Pyrex test-tube. Any iron present is de-ionised with ammonium citrate or pyrophosphate, and a solution of diethyl-dithiocarbamate is added, followed by amyl alcohol, all these operations being carried out in the Pyrex tube. After shaking, about 3 or 4 ml. of the amyl alcohol extract are withdrawn and the colour compared with standards. No mention is made of bismuth which, if present, may affect the colour of the amyl alcohol extract. The possible presence of bismuth due to bismuth medication should always be considered when examining human biological material.

Moir and Andrews (1940) give a method for determining traces of copper in butter, in which the butter is melted and the aqueous portion treated with hydrogen peroxide, hydrochloric acid, trichloracetic acid and sodium tungstate. The filtrate after addition of sodium citrate is made alkaline with ammonia, sodium diethyl-dithiocarbamate added and the liquid extracted with amyl alcohol. The colour of the amyl alcohol solution is compared with that of standards. It is claimed that, working on 25 g. of butter, it is possible to determine less than 0·1 p.p.m. of copper, and that with careful working the blanks do not exceed 0·05 to 0·09 p.p.m.

Interference by iron and other metals and by calcium phosphate may be avoided by combining the dithizone extraction of copper from acid solution with its determination by diethyl-dithiocarbamate. The following method has been found by me to give satisfactory results with most types of food.

A GENERAL METHOD FOR COPPER IN FOOD. A suitable weight of the sample, usually about 10 g., is mixed with 5 ml. of sulphuric acid, and nitric acid added as required in small quantities at a time, the mixture being heated until all organic matter has been destroyed without charring. The liquid is freed as far as possible from nitric acid by diluting with about 20 ml. of water and heating again until the sulphuric acid fumes freely. If the sample contained much fat the acid residue should be heated with ammonium oxalate (p. 85).

After cooling, the liquid is diluted with water and 2 g. of citric acid added. It is neutralised with ammonia, re-acidified with 1 ml. of concentrated hydrochloric acid, cooled and transferred to a separator. The volume with washings should measure about 100 ml.

Copper is now extracted by shaking with three successive quantities of 5 ml. of a freshly prepared 0·1 per cent solution of dithizone in chloroform. The shaking must be violent and continued for at least a full minute for each extraction. Each of the three extracts is washed in turn with about 10 ml. of water in a small separator. The chloroform layers are transferred to a 6″ × 1″ Pyrex test-tube and the chloroform evaporated in a hot-water bath.

The residue containing copper dithizone is heated in the test-tube with 1 ml. of sulphuric acid and a little nitric acid until organic matter has been destroyed. About 5 ml. of water is then added and the liquid boiled until the residual sulphuric acid fumes freely. It now measures about 0·8 ml. and contains the whole of the copper. It is diluted with water and the whole, or a measured part, according to the amount of copper present, transferred to a 50-ml. Nessler cylinder. 1 ml. of 5 per cent citric acid solution, 4 ml. of 0·880 ammonia and 5 ml. of 1 per cent gum arabic solution are added, and the contents of the cylinder made up with water to the 50 ml. mark and mixed. 5 ml. of a freshly prepared 0·2 per cent solution of sodium diethyl-dithiocarbamate are added and the colour matched by adding a standard copper solution (1 ml. = 0·01 mg.) to a control cylinder containing similar quantities of reagents as are present in the test solution.

If, as occasionally happens, the hues of the control and of the test solution are different, making an exact match difficult, the copper complex is extracted from the test solution with chloroform and the extract made up to 10 or 20 ml. with chloroform. The colour of the solution is then measured in a Lovibond tintometer and the copper corresponding to the yellow units found from a graph of the results obtained by extracting known amounts of copper (Haddock and Evers 1932). Alternatively the optical density of the solution can be determined in a photometer.

With materials containing very small amounts of copper, of the order of 1 p.p.m., the greatest care must be taken to avoid accidental contamination with traces of copper, attention being paid to such details as the use of gas burners fitted with iron tubes instead of brass. Traces of copper are often present in distilled water. A blank experiment is performed on the apparatus and reagents with each set of determinations. This blank should be carried right through all the analytical operations in a manner precisely similar to that of the actual determination.

Strafford and others (1945) use diethylammonium diethyldithiocarbamate instead of the sodium salt, since it offers several advantages such as ease of preparation (from diethylamine and carbon disulphide), high stability and completeness of reaction with copper (cf. p. 188).

In all colorimetric work with copper it should be borne in mind that very dilute standard copper solutions lose strength by precipitation of copper on the surface of glass unless they contain a small amount of free sulphuric acid.

POLAROGRAPHIC METHOD. The so-called 'polarographic' method of determining traces of metals, etc., in solution was introduced by Heyrovský in 1925. It depends upon the fact that different metallic cations require the application of different electrical potentials before they are deposited from solution upon a cathode. If the potential between the solution and the cathode is gradually raised and the values plotted against the current, the resulting voltage-current graph shows upward turns at the points at which sudden increases of current occur due to decomposition of the electrolyte and discharge of metallic ions at the cathode. To avoid complications due to polarisation of the electrodes, Heyrovský used a dropping mercury cathode and a large mercury anode. The mercury forming the cathode emerges from a vertical capillary tube at the rate of one drop every three seconds, and the surface of the cathode is thus constantly being renewed. Polarisation conditions are therefore kept practically constant, and owing to the high hydrogen overvoltage of mercury there is no evolution of hydrogen from the cathode (cf. p. 174). The extent of displacement of the curve at the points of inflexion of the voltage-current graph can be used to measure the amount of metal present provided that the apparatus is calibrated for this purpose. The decomposition potentials of different metals in solution must be wide enough apart to make a distinction possible, and various devices may be employed to distinguish the cations responsible for the inflexions of the current-voltage graph such as altering the reaction of the solution or forming complex ions with potassium cyanide. The method is sensitive but has many limitations and requires careful calibration and adjustment for each different kind of sample. It is specially suited to the routine determination of metallic impurities in commercial products, and might be particularly useful for determining metals in the solution obtained after wet oxidation of organic material. A review of the principles of the method is given in the *Chemical Society Annual Reports*, 1933, **30**, 268–270 ; 1938, **35**, 389–394 ; (cf. also Gaskin and Whalley 1943, Davies 1946, Heyrovský 1947, and several other papers in the *Analyst* for 1946 and 1947). The apparatus is complicated and expensive, but Davies (1941) suggests that a cheaper type for manual operation can be made up in the laboratory from standard parts. A useful account of the application of polarographic analysis to the determination of toxic metal fumes (lead, zinc, cadmium, chromic acid and manganese) in air, applicable with modifications to foods and biological material, is given by Levine (1945).

OTHER METHODS OF DETERMINING COPPER IN FOOD. Several other methods for the determination of copper in food and biological material have been described, of which the following are a selection. There are also a great many colour and precipitation tests designed to detect the presence of copper in small amounts of material :

1. Colorimetric determination as the blue cupric ammonium compound (Hanak 1930).
2. Colorimetric determination as cupric sulphide.
3. Reduction to cuprous oxide with glucose, oxidation of the cuprous oxide with ferric sulphate and titration with permanganate (Astruc and Castel 1935, von Fellenberg 1932).
4. Precipitation of metallic copper with zinc dust followed by colorimetric determination (Kobljanski 1934).

5. Precipitation with benzoin-oxime (Gruzewska and Roussel 1938).

6. Precipitation with α-nitroso-β-naphthol and ignition to cupric oxide (Gruzewska and Roussel 1935).

7. Colorimetrically with ammonium thiocyanate and toluidine, a variant of the Biazzo method (Lirtzman and Kulberg 1936).

8. Direct precipitation from wine or cider with tannin and ferrocyanide solution with subsequent separation as sulphide (von der Heide 1925).

9. Titrimetric determination with nitroso-chromotropic acid (1:8-dihydroxy-2-nitroso-3:6-naphthalene disulphonic acid) (Cherbuliez and Ansbacher 1930, Ansbacher and others 1931).

10. Colorimetric determination with dithio-oxamide or rubeanic acid (Allport and Skrimshire 1932).

11. Microgravimetric determination with salicyl-aldoxime (Reif 1931, Austin and Riley 1933).

12. Colorimetrically with ammonium persulphate, silver nitrate and dimethyl-glyoxime (Hurd and Chambers 1932, Clarke and Jones 1929).

13. Spectrographic methods (Lewis 1935, Twyman 1937).

14. Determination by catalytic oxidation of cysteine to cystine, and also of thio-acids (Warburg 1927, Bjerrum 1936).

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2

[LEAD]

LEAD is one of the most toxic of the trace elements occurring in food. Its effects are cumulative in that repeated doses, each of which is far too small to produce any appreciable effect on health, may in course of time cause serious symptoms of poisoning.

As far as is known lead is not an essential constituent of any living organism. It gets into food partly from traces naturally present in soil, but mainly from the use of the metal and its alloys or compounds in plant and vessels in which food or the raw materials of food are manufactured, transported or stored, for instance lead linings, terne plate, soldered joints, lead pipes, lead glazes and enamels. Its occurrence in food may also be due to the use of lead arsenate as an insecticide spray for fruit and vegetables and to a less extent to dust produced by the weathering of lead paint. Shell-fish and crustacea may absorb lead from sea-water, especially from estuary water contaminated with town drainage and factory effluents.

THE NORMAL DAILY INTAKE AND EXCRETION OF LEAD.

From Food. Determinations of the lead content of foods comprised in several common dietaries, combined with records of the amount of each food ordinarily consumed, have shown that the mean daily intake of lead in food by an adult is between 0·2 and 0·25 mg. This does not include water, which in some districts may contribute an appreciable amount of lead derived from lead pipes. Ingleson's tests (1938) on nine domestic water supplies in different parts of the country showed an average lead content of 0·118 p.p.m. Taking 3 pints as the mean daily consumption of water per head for cooking and drinking (Magee 1937), this corresponds to a daily intake of 0·2 mg. from this source alone, but it is doubtful whether the mean of so few samples can be taken as representative of the general run of drinking-water. Probably 0·1 mg. daily would be nearer the mark, giving a mean total intake of a little over 0·3 mg. from food and water alone.

From Inhaled Lead. In the course of work carried out in the Government Laboratory for the Departmental Committee on Ethyl Petrol (1930) it was found that the average amount of lead inhaled as dust in the air of towns was 0·077 mg. daily. The figures will, of course, vary widely for different places and for different seasons of the year, but over a long period it may be taken that the daily intake of lead from this source is about 0·08 mg. How much of this reaches the lungs and how much is retained in the nose and throat and eventually finds its way into the alimentary tract it is difficult to say. In any case, however, it brings up the total mean daily intake of lead from all sources to about 0·4 mg.

Urinary Excretion of Lead. The Departmental Committee on Ethyl Petrol found that the excretion of traces of lead in urine is the rule and not the exception. Practically everyone excretes small quantities of lead through the kidneys, quantities of the order of 0·05 mg. daily. Figures given by various authorities range from 0·04 to 0·08 mg. It appears therefore that the daily excretion by the kidneys may roughly balance the amount inhaled in the form of dust. Lead which reaches the lungs must pass into the general circulation as there is no other path open to it, and from the blood-stream the most probable path of excretion is by way of the kidneys. It was these considerations, no doubt, which led the Departmental Committee to express the opinion that the presence of lead in the dust of towns is one of the causes, if not the main cause, of the almost universal excretion of lead in normal urine.

Up to quite recent times it was believed that the presence of lead in urine was a sure sign of lead poisoning. Since the discovery that traces are almost always present in normal urine it has been held that the excretion of more than 0·1 mg. daily indicates lead poisoning. This estimate, however, is often misleading. In severe lead poisoning urinary lead is about 0·15 mg. daily, but it may be much less than this or even no higher than normal. On the other hand, 0·2 mg. may be excreted daily in urine without symptoms of poisoning. Lead workers seem to be in a class by themselves. They can excrete much larger amounts of lead both in urine and faeces than normal persons without apparent ill effects, and no satisfactory explanation of this anomaly has been forthcoming. It seems that with constant exposure to lead hazards a high degree of tolerance may be established.

According to Wood (1941) nearly all urinary calculi contain appreciable quantities of lead.

Faecal Excretion of Lead. It is more difficult to arrive at an average figure for faecal lead excretion than for urinary excretion owing to irregularity of distribution. Kehoe and others (1933) give the mean daily excretion in faeces as 0·3 mg. and Tompsett and Anderson (1935) 0·22 to 0·4 mg. The amount of lead thus excreted is therefore about six times as great as in urine and is roughly equal to the amount ingested with food and water. While the above may be taken as average figures, great differences are found in the amounts excreted by normal persons. According to Kehoe, the daily excretion in faeces may rise to 0·6 mg. before any risk of poisoning need be apprehended, but 1 mg. is usually associated with poisoning (cf. also Tompsett and Anderson 1939). Perhaps in view of Kehoe's later work (p. 82) the degree of tolerance should be placed even higher. It is held by some authorities that the upper limit of normality is 5 mg. of lead in 100 g. of dried faeces, which would represent 1·5 to 1·75 mg. of lead daily.

RETENTION OF LEAD IN THE BONES. It has been known since the middle of last century that the bones contain more lead than any other part of the body, but this fact appears to have been lost sight of for some time, and it was not until Minot's and Aub's work in 1924 and subsequent years that the extent of the storage of lead in the bones was generally realised. The amount of lead in bones varies greatly. Plate bones contain more than tubular, the femur and tibia more than the ribs and vertebrae, and the ends more than the shaft. Roche Lynch and others (1934) found from 15 to 140 p.p.m. of lead in different bones, all from healthy subjects, and rather more in teeth. They point out that it is quite possible for a man in apparently normal health to have 100 p.p.m. of lead in his skeleton, equivalent to 1·5 g. in his whole body. The average amount in the bones of a middle-aged man may perhaps be taken as about 50 p.p.m. or 0·75 g. in the whole skeleton. This would imply a mean rate of storage of about 0·05 mg. a day, but it may be noted that Hansmann and Perry (1940) could find no relationship between lead in the bones and age of the subject.

Lead stored in bones is not necessarily inert. Circumstances may arise in which it is gradually released from the bones into the blood-stream, where it may cause lead poisoning. Any conditions which favour decalcification of bone will also favour the release of lead (Aub and others 1926), and where there is a deficiency of calcium in the food, lead poisoning is more likely to occur (cf. also p. 72). Certain drugs such as phosphoric acid and ammonium chloride which produce acidosis, and also potassium iodide, are reputed to have this effect (cf. Tompsett 1939). This is the basis of the de-leading process which is often resorted to in cases of plumbism (Hunter 1944), but according to Kehoe and others (1943) it is very doubtful whether such treatment materially hastens the elimination of lead. Aub suggests that lead phosphate, which is the form in which lead is deposited in bone, is least soluble within the normal range of acidity of the organism and that variations above or below this degree of acidity favour solution and eventual elimination of lead. Roche Lynch (1934) and Weyrauch (1935) suggest that shock, such as may occur after an operation, acute infections, alcoholism, starvation or even changes in diet, may have the effect of ' mobilising ' lead from the bones and liberating it into the blood. Sobel and others (1940) discuss the rather complicated relations believed to exist between vitamin D, phosphorus and calcium intake and the deposition of lead in bones. The isomorphism of calcium and lead in apatite and in pyromorphite (lead chlorophosphate) is of interest and possibly of importance in this connection, since the inorganic part of bone consists largely of hydroxyapatite (p. 348).

LEAD BALANCE IN NORMAL PERSONS. From the figures given above we may draw up an approximate balance sheet showing the daily intake, storage and excretion of lead in a normal healthy individual, as follows :

Intake		*Excretion and Storage*	
From food . . .	0·22 mg.	Faeces	0·30 mg.
From water . . .	0·10 mg.	Urine	0·05 mg.
From inhaled dust .	0·08 mg.	Stored in bones . .	0·05 mg.
	0·40 mg.		0·40 mg.

These figures are a very rough approximation, but they probably give a fair indication of the normal intake and excretion of lead.

LEAD IN BLOOD. To exert its full toxic effect lead must gain access to the systemic blood, and the lead content of blood is therefore the best index of lead poisoning. The lead content of blood is normally about 0·2 p.p.m. and in cases of lead poisoning 0·6 to 0·7 p.p.m. Litzner and Weyrauch (1933) give the following limits :

0·1 to 0·3 p.p.m. .	not significant except possibly in tissues already damaged by lead.
0·3 p.p.m. . .	symptoms of poisoning may begin.
0·6 to 0·65 p.p.m. .	critical level usually associated with onset of severe symptoms.
0·7 to 1·0 p.p.m. .	almost always severe symptoms of poisoning.

Other investigators have found 0·7 to 0·9 p.p.m., and even more, in the blood of persons not apparently suffering from lead poisoning, but from a summary by Willoughby and Wilkins (1938) it would seem that 0·2 to 0·3 p.p.m. is a fair average. Kaplan and McDonald (1942) give a normal average of 0·3 p.p.m. Tracy and McPheat (1943), as a result of spectrographic determinations, give a range of 0·05 to 1·2 p.p.m. with a mean of 0·4 p.p.m., and regard 1·3 p.p.m. as the critical concentration for lead poisoning, i.e. twice as much as Litzner and Weyrauch.

The toxic action of lead in the blood-stream is characterised by anaemia, ascribed by some to a progressive destruction of the blood-cells by the deposition of insoluble lead phosphate on their surface and local production of acid, which makes the cell wall brittle and easily disrupted (Rodgers and others 1934). The blood also serves as a vehicle for carrying lead to various tissues of the body, where it causes damage and is then removed by the blood-stream and either excreted or stored in the skeleton (Roche Lynch 1934, Fairhall and Miller 1941). Apparently lead does not accumulate to any great extent in the liver, kidneys or other organs of the body, although this may happen in certain cases of chronic plumbism (Monk 1933).

INTESTINAL ABSORPTION OF LEAD. The amounts of lead normally eliminated in urine and faeces are roughly equivalent to those taken in by the lungs and the alimentary tract respectively. This might suggest that it is only lead inhaled as dust which finds its way into the circulation and that lead ingested with food is excreted without absorption. In fact, the view is widely held that industrial lead poisoning is due almost entirely to inhaled lead. It is probable, however, that even with the normal daily ingestion of only 0·3 mg. there is a certain amount of absorption from the intestinal tract and a corresponding excretion from the general circulation into the tract.

It is not easy to differentiate between lead which is passed through the intestinal tract unabsorbed and lead which is absorbed and re-excreted into it. With large doses there is, of course, considerable absorption, and the probability is that lead is also excreted more or less throughout the whole length of the alimentary canal. That it is excreted from the liver by way of the bile is evident from the observation of Aliavdin and Peregood (1936) that the duodenal juice of hospital patients with suspected lead poisoning contained 10 to 30 times as much lead as their urine (cf. also Anderson 1945). The work of Behrens (1925, 1933), Miyasaki (1930) and Fees (1932) on mice also shows that lead in certain circumstances may be freely absorbed from the intestinal tract and excreted into it again (cf. also Minot 1924).

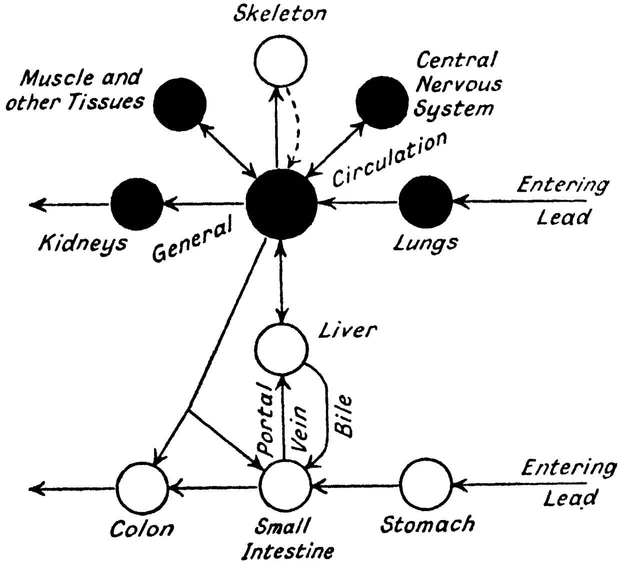


DIAGRAM SHOWING ABSORPTION AND EXCRETION OF LEAD

(*Cf.* Aub, Fairhall, Minot and Reznikoff: *Lead Poisoning*; Baltimore, 1926.)

Black circles represent tissues and structures easily damaged by lead; white circles those which are comparatively resistant.

The accompanying diagram adapted from those given by Aub (1926) shows the probable path of lead in the body when ingested and when inhaled. The black circles represent tissues and structures readily damaged by lead and the white circles those which are comparatively resistant. When lead is absorbed from food in the intestine it is carried to the liver by the portal vein. Some of this lead is re-excreted in the bile without ever reaching the general circulation or vulnerable tissues. Some of it, on the other hand, may get into the general circulation from the liver and may cause lead poisoning, being subsequently excreted (i) through the kidneys, (ii) through the liver and bile, or (iii) through the walls of the intestinal tract. When lead is inhaled as dust into the lungs it must all pass into the general circulation where it is actively toxic. It is then excreted by the same paths as if it had reached the general circulation from the alimentary tract. Weyrauch (1930) considered that the liver is highly efficient in removing lead from portal blood but not so efficient in removing it from the general circulation.

EFFECT OF FOOD ON INTESTINAL ABSORPTION OF LEAD. Most outbreaks of lead poisoning in recent years have been caused by lead in water, beer or cider and comparatively few by lead in solid foods. Beer and cider are usually consumed in much greater quantity than solid food, and moreover it is clear that, in general, lead is more readily absorbed from liquids than from solids. Jackson (1932) found that cider which was suspected of having caused lead colic contained from 0·7 to 1·4 p.p.m. of lead, and his figures indicate that the daily intake of lead from this source ranged from 1 to 5 mg. and averaged about 2·5 mg. Turning to solid foods, we find that six sardines containing 30 p.p.m. of lead, a not uncommon amount some years ago, would also furnish 2·5 mg., yet no instances of lead poisoning from sardines are on record. Different kinds of solid food may affect absorption to different extents. Miyasaki (1930), in experiments on mice, found that oats and bran interfered only slightly with lead absorption, but that milk had a much greater effect. Milk is one of the antidotes sometimes administered for acute lead poisoning. Horwitt and Cowgill (1939), in experiments on the feeding of lead to puppies, found that on a dried milk diet very little lead was retained, while on a mixed diet retention was much greater. The calcium-phosphorus ratio in the food seems to be an important factor, and in milk it is probably the colloidal calcium phosphate that immobilises lead. Tompsett (1939), working with mice, found that with high-calcium diets lead absorption was low and with low-calcium diets lead absorption was high. The addition of a small quantity of hydrochloric acid to a high-calcium diet was found to raise the absorption of lead in certain circumstances by over twenty times. Fats and vitamin D were without influence.

Like many other heavy metals, lead forms complexes with some of the products of protein hydrolysis and with organic bases, and these may be insoluble in the intestinal digestive juices or not readily dialysed through the epithelial membranes. Again, in certain foods with a high sulphur content lead may be precipitated as sulphide in the alimentary tract, in which condition it is insoluble and non-toxic. Miyasaki (1930) considered that intestinal absorption of lead is a simple diffusion process depending on the relative concentrations of soluble lead in the blood and in the intestinal tract. Tompsett (1940) found that lead did not dialyse readily through parchment from suspensions of undigested egg-white or egg-yolk, but that it dialysed more easily from these foods when acidified or from a peptic digest of them at pH 4·2. When the peptic digest was neutralised with sodium carbonate dialysis was again inhibited.

Lead forms an insoluble phytate, and the presence of phytic acid in cereal foods might be expected to interfere with its absorption from the intestine, although Miyasaki's results with oats and bran, referred to above, do not indicate this. Murer and Crandall (1942) have found that pectin forms an insoluble compound with lead and interferes with its absorption. In presence of pectin more of the ingested lead passes through to the faeces and less is excreted in the urine. This may be of significance in connection with spray residues on fruit.

The question whether and to what extent food interferes with intestinal absorption of lead is obviously of great importance in connection with permissible limits for lead in different foods. Similar considerations apply to other toxic metals.

THE CONTAMINATION OF FOODS WITH LEAD

Lead Glazes and Alloys. In this country nearly all pottery cooking ware is made with leadless glaze, but a few of the smaller potteries still use lead glazes, and many lead-glazed casseroles and dishes are also imported in normal times, mostly from France. The solubility of lead glazes in dilute acids was investigated by Thorpe (1901) and again inquired into by a Departmental Committee in 1908. The investigations were concerned mainly with the health of pottery workers and with the solubility of lead silicate dusts in gastric juice. They were not extended to the solubility of lead glazes in foods, but the conclusions arrived at have an intimate bearing upon food contamination. Thorpe found that the solubility of fritted glazes in dilute acids depends upon the ratio of basic molecules to acid molecules in the material. If the ratio of the total number of acid molecules to the total number of basic molecules is 2 or more, the resistance to corrosion by acids is high. Again, if the total weight of acids calculated as silica is 1·85 times the total weight of bases calculated as lead oxide, the resistance is high. The higher the silica content, as a rule, the less brilliant the glaze and the higher the temperature required to fuse it, so that the tendency is to use low-silicate, easily fusible glazes and these are the more readily corroded. In an investigation into glazes and enamels of cooking vessels (Monier-Williams 1925) it was found that foods cooked in lead-glazed earthenware might take up from 3 to 4 p.p.m. of lead, and hot dilute citric acid solution, allowed to simmer in these vessels for half an hour, as much as 80 p.p.m. (cf. Masters 1919, 1920).

In Germany, glazes and enamels are condemned if they yield soluble lead on being heated for half an hour with 4 per cent acetic acid. Apparently the standard adopted in practice is 2 mg. of lead per litre of capacity. The acid under the conditions of the German test takes up very roughly ten times as much lead as food cooked in contact with the same glaze. In this country there are no regulations under which lead-glazed cooking vessels can be dealt with.

Pewter, a tin-lead alloy containing varying amounts of lead from 7 per cent upwards, is not now much used. It was formerly believed that the addition of tin to lead prevented it from dissolving, but all tin-lead alloys give up small amounts of lead to food, some being more resistant than others. Matthews (1929) recommends that lead pipes used for beer should contain 15 per cent of tin to lessen the risk of lead contamination. Dunn and Bloxam (1930) found that kettles are sometimes tinned with an alloy of one part of tin and two parts of lead (cf. ' terne ' plate, Price 1945), and tin containing about 4 per cent of lead has been used for the plating of some of the cheaper qualities of pressure cookers. Small amounts of lead have also been found in the tin-foil used for wrapping processed cheese. In several Continental countries solder for food receptacles is not allowed to contain more than 10 per cent of lead, and tin plating of cans and cooking vessels more than 0·5 to 1 per cent.

Lead in Water. In a series of papers in the *Analyst* Thresh has described investigations on the action of natural waters upon lead, and his results may be summarised as follows :

> The presence of oxygen is essential, and the first action is the formation of lead oxide upon the surface of the metal. In water containing carbonates the lead oxide film is converted into insoluble oxycarbonate. If the water contains very little carbonate some of the lead oxide is dispersed before becoming converted into oxycarbonate, and the water becomes turbid. This is the so-called ' erosive ' action. If the water contains free carbonic acid as well as carbonate, the lead carbonate remains in solution until no more free carbonic remains.
>
> Silicates, even to the extent of only 1 part in 100,000, completely prevent the surface oxidation of lead, and the metal becomes coated with an insoluble protective film of silicate. Soft waters containing free acid dissolve lead oxide, forming a lead salt in solution.

The action of water on lead has lately been re-examined in somewhat greater detail by Liddiard and Bankes (1944).

Ingleson's tests (1938) on a number of domestic water supplies have already been referred to (p. 64). They were carried out by filtering water, drawn for household use, through a layer of magnesium oxide and calcium carbonate which takes out all the lead. The total amount of water passed through the filter was measured by a meter. After a period of several weeks, lead in the filter material was determined and calculated to parts per million of water. With nine water supplies in different parts of the country, sampled over different periods of time and at different seasons of the year, the results varied from 0·005 to 0·5 p.p.m. and averaged about 0·1 p.p.m.

The question of permissible limits for lead in domestic water supplies is referred to on page 81.

Aerated water, owing to its high content of carbonic acid, is specially liable to dissolve lead if brought into contact with it. Henderson (1927) found that the soft water of Brisbane when carbonated and sold from soda-water fountains made with soldered fittings, or plated with tin containing lead, took up relatively large quantities. Two-fifths of all the samples examined contained more than 0·7 p.p.m. ($\frac{1}{20}$ gr. per gallon), the highest being as much as 16 p.p.m. (1·1 gr. per gallon). In this country several samples have been reported in recent years as containing from 0·7 to 20 p.p.m.

It is probable that potatoes and other vegetables, when boiled in water containing lead, take up much of the lead present (Seiser, Necke and Weber 1942).

Cider and Beer. Cider has on several occasions become seriously contaminated with lead owing to its solvent action on lead pipes and on the lead fittings which were at one time common in cider presses. Lead pipes are still in use in country districts, and cases occur of 'Devonshire colic', which is in fact lead poisoning. Many of the so-called tin-lined pipes used to bring cider and beer from the cellar to the bar are made of lead with a very thin internal coating of tin

(' tin-washed ' pipes). In making lead pipes, lead at a temperature close to its melting-point is forced by a hydraulic press upwards through a die and forms a continuous pipe which is wound on a drum. To produce a tin-washed pipe, molten tin is poured from a ladle into the open end of the pipe as it emerges from the press. The tin, since its melting-point is lower than that of lead, stays liquid inside the hot pipe and runs down it, leaving a very thin film of tin on the inner surface. This film is not thick enough to protect the lead from corrosion for long and soon becomes worn away in use. The true tin-lined pipe has a lining of block tin at least a millimetre thick. It is actually a tin pipe with an outer casing of lead, and of course is much more expensive than a tin-washed pipe (*Ann. Rept. C.M.O.*, 1932).

First-drawn cider which has lain in the pipes all night is the most liable to contain lead. This first-drawn cider should be thrown away, but there is reason to believe that it is frequently returned to the cask.

Lead pipes are often used for beer. Since beer is not so acid as cider, lead poisoning from this cause is of rarer occurrence. In two cases of poisoning with men whose daily intake of beer was a gallon or more, samples of the beer were found to contain varying amounts of lead up to 2·8 p.p.m. Lead poisoning has also been caused by beer which had been kept in lead-glazed tanks (*Ann. Repts. C.M.O.*, 1922, 1936).

Canned Foods. Canned foods are now less liable than in past years to contain lead owing to technical improvements in canning, which have reduced greatly the amount of lead solder in contact with the food. Canned beef has been found to contain up to 5 p.p.m. and occasionally more. A good deal of trouble has been caused in recent years by sardines, which are cooked on grills before being canned. These grills are often coated with an alloy of lead and tin, and the sardines may take up as much as 90 p.p.m. of lead (Lampitt and Rooke 1933). In 1936 medical officers of health at the ports agreed to take action after July 1937 in respect of all consignments of imported sardines showing more than 5 p.p.m., with a view to reducing this limit at a later period to ' negligible traces ' of lead (*Ann. Repts. C.M.O.*, 1936). Cheftel and Pigeaud (1937) claim that with every care to exclude lead it is almost impossible to reduce it below 3 p.p.m. and suggest a limit of 5 p.p.m. Canned brisling and silds are, however, produced with less than 1 p.p.m. of lead, and it should be possible to do the same with sardines.

Baking Powders and Self-raising Flour. Acid calcium phosphate often contains lead owing to its having been made by the action of sulphuric acid on bone ash or rock phosphate in leaden vessels. According to Fox (1922) it may contain from 200 to 2,000 p.p.m. of lead which would mean that self-raising flour made with phosphate might contain from 2 to 20 p.p.m. Acid calcium phosphate can easily be obtained with a guaranteed content of less than 10 p.p.m. of lead.

Tartaric acid, citric acid and cream of tartar were at one time liable to be contaminated with lead derived from leaden pans used for concentrating and crystallising the acids, but it is unusual nowadays to find them with more than 2 or 3 p.p.m. In a Local Government report published in 1907 MacFadden suggested a limit of 20 p.p.m. for lead in these products, and this was adopted in the British Pharmacopœia of 1932. In view of improvements which have taken place in manufacturing processes it would seem that this limit is too high and could with advantage be reduced.

Food Colours. The contamination of food colours with lead was investigated in 1935 by a sub-committee of the Analytical Methods Committee of the Society of Public Analysts. Most of the colours examined contained less than 20 p.p.m., but a few showed much more, up to 400 p.p.m. in ferric oxide (Armenian bole). Damson blue, a mixture of colours sold retail for use in food, was recently found to contain as much as 540 p.p.m. It would seem that most, if not all, of these colours can be produced with a lead content of not more than 20 p.p.m. The Departmental Committee on Preservatives and Colouring Matters in Food (1924) recommended a limit of 20 p.p.m. for lead, copper, tin and zinc together in artificial food colours. Ferric oxide used for colouring food should contain no lead, as it can be made by calcining pure recrystallised ferrous sulphate.

Lead chromate was referred to by Hassall in 1876 as being used for the colouring of curry powder. The existence of this reprehensible practice seems to have been completely lost sight of in subsequent years until rediscovered, apparently still flourishing, in 1933, when samples were found to contain from 20 to 160 p.p.m. of lead. The lead was eventually traced to turmeric, an invariable ingredient of curry powder. It is customary in some countries where turmeric is grown to treat it with 'lemon chrome' powder to improve its colour. One sample of turmeric root contained 282 p.p.m. and the yellow powder on the surface as much as 0·5 per cent of lead (*Ann. Repts. C.M.O.*, 1933, 1934, 1935). The presence of chromium was confirmed by the diphenylcarbazide test (p. 441).

Tea. Tea imported in lead-lined chests usually contains from 10 to 20 p.p.m. of lead and sometimes more, in the form of dust abraded from the inner surface of the lining. Contamination is lessened but not entirely prevented by paper linings inside the lead sheet. About one-third of the lead present in the dry tea passes into the infusion, but owing to the large quantity of infusion compared with the weight of tea the actual concentration of lead in the infusion is small. Tea containing 20 p.p.m. of lead gives an infusion containing about 0·15 p.p.m., which is well within the limit usually accepted as safe for domestic water supplies. A small amount of lead, of the order of 2 p.p.m., seems to be unavoidable in tea, being picked up during the various processes to which tea is subjected and becoming more concentrated as the leaf loses weight in drying. The United States limit for lead in imported tea is 2·5 p.p.m.

Milk. The use of lead foil or tinned lead caps for milk bottles has been shown by Kerr (1942) to cause serious contamination of the milk with lead. In one series of tests bottles of milk were closed with lead foil caps coated with 0·00012 inch thickness of tin. After twelve hours the cream taken from the milk was found to contain from

4 to 6 p.p.m. of lead. If the milk was kept for 48 hours, by which time it had soured, the lead content of the cream was from 7·5 to 16 p.p.m.

Lead may pass into the milk of cows suffering from lead poisoning (White and others 1943) which is sometimes the result of licking the paint off gates and railings.

Agricultural Insecticides. Lead arsenate is widely used as an insecticide for fruit and vegetables. It is usually sprayed on to the trees as a suspension in water mixed with various ‘ spreaders’, which assist the production of a uniform coating on the fruit and also prevent it from being easily washed off by rain. According to Frear and Worthley (1937) the minimum amount of lead arsenate necessary for protection of fruit is 125 mg. per square metre of fruit surface. This would, with apples, correspond very roughly to .6 p.p.m. of lead. Sprayed apples have been found to contain up to 20 or even 40 p.p.m. In recent years greater care has been taken to wash sprayed fruit in dilute hydrochloric acid to remove excess of lead arsenate before marketing, and both lead and arsenic are generally reduced to reasonable amounts. The United States Federal limit for lead in sprayed fruit was at one time 0·018 grains per pound (2·6 p.p.m.), but was raised in 1938 to 0·025 grains per pound (3·6 p.p.m.) and in 1941 to 0·05 grains (7·2 p.p.m.). Vegetables also are sprayed with lead arsenate ; Henderson (1929) found as much as 1 gramme (15 grains) in a cabbage.

Lead arsenate as prepared from lead acetate and arsenic acid has the formula $PbHAsO_4$. A mixture of basic lead arsenates with nominal formula $Pb_3(AsO_4)_2$ is also used, but not so widely as the acid arsenate (Freak 1942).

Lead arsenates, owing partly to their insolubility, are considerably less toxic to man than other lead and arsenic compounds. Fairhall (1939) suggests that in contact with body fluids lead arsenate may form the insoluble compound $Pb_4(PbCl)(AsO_4)_3$, and that for this reason it is not so toxic as might be expected (cf. pp. 164, 167). Fairhall and Neal (1938) gave 100 mg. of lead arsenate to two individuals over a period of ten days on a controlled diet. This is about

6 to 7 mg. of lead and 2 to 3 mg. of arsenic daily. No ill effects were observed, but ten days seems rather a short time in which to expect conclusive results with this dosage. Similar results are recorded by Webster (1941). Shields, Mitchell and Ruth (1939) added lead arsenate to the food of rats and found that even with as much as 144 p.p.m. of lead in the food for eight to seventeen weeks the growth and apparent health of the rats were not affected. Rats, however, are very tolerant both to lead and to arsenic. Under the influence of weathering the ratio of lead to arsenic in sprayed fruit increases, indicating the conversion of some of the lead arsenate to other compounds in which the lead may not be so insoluble (cf. p. 167). Apart from solubility there is no doubt that arsenates, at certain levels of intake, are less toxic than arsenites owing to the fact that they do not combine with thiols in the body. This point is further discussed on page 163.

In wine made from sprayed grapes 5 to 12 p.p.m. of lead has been found, but according to Kielhöfer (1929) lead from spray residue is mostly removed by yeast during fermentation, and it is unusual to find more than 1 p.p.m. in wine. Storage appears to reduce the lead content, probably through combination with proteins and precipitation. Wines which originally contained from 3 to 8 p.p.m. were found by Dresel (1927) to contain only 0·4 p.p.m. after four months' storage.

Commercial pectin preparations made from apple pomace and citrus fruit waste are liable to contain small amounts of lead.

Shell-fish. Chapman (1926) found from 80 to 100 p.p.m. of lead in oysters, and smaller amounts, up to 7 p.p.m., in other shell-fish and crustaceans. This lead was derived apparently from estuary water. Water from the Thames estuary contained from 0·12 to 1 p.p.m., and it is probable that mussels and oysters, which pass large quantities of water through their gills, may be unable to prevent the absorption of lead and may have become inured to its presence. The same would apply also to copper (p. 13) and zinc (p. 113).

LEAD AND PLANTS. Soil can absorb and immobilise lead, so that fairly large amounts may be present without causing injury to crops (Keaton 1937). In water cultures, 0·1N solutions of lead salts are toxic to plants, but more dilute solutions are harmless (Hevesy 1923). Crops grown on soils containing lead do not as a rule take up enough to raise significantly the total lead intake in food. In this connection, however, recent work by Kent (1942) should be noted, which seems to indicate that the natural lead content of wheat may be much greater than previously suspected. Using a spectrographic method, he finds 0·9 p.p.m. of lead in wheat flour and 3 p.p.m. in bran. The pericarp of the wheat ('beeswing') contained as much as 4·8 p.p.m. The spectrographic method also showed similar values for tin in wheat products, and smaller values, but still unaccountably high, for silver. These results are much higher than those obtained by chemical methods and must perhaps be accepted with reserve in their application to the general run of wheat products, pending confirmation by further work on a wider range of samples (cf. p. 97).

LIMITS FOR LEAD IN FOODS. In this country there are few statutory limits for lead in food. Under the Preservatives Regulations 1925–7 the use of any compound of lead, e.g. lead chromate, for colouring food is prohibited. Compounds of chromium are also prohibited so that lead chromate is under a double ban. Under the Edible Gelatin (Control) Order, 1947, a limit of 10 p.p.m. has been prescribed for gelatin. With these exceptions the contamination of food with lead is governed only by the general provisions of the Food and Drugs Act of 1938, in particular by Section 1 of this Act which prohibits the addition of injurious substances to food.

For domestic water supplies the view is widely held that 0·3 p.p.m. is safe, 0·5 p.p.m. the maximum permissible amount and 0·7 p.p.m. on the dangerous side. Taking the daily consumption of water as 3 pints per head (Magee 1937), these figures represent intakes of 0·5, 0·85 and 1·2 mg. respectively. They must be regarded as additional to the normal lead intake from food and inhaled dust together, which has been roughly estimated (p. 68) at 0·3 mg. If we assume that lead, at these low levels of intake, is absorbed from food as readily as it is from water, we arrive at 0·8 mg. as a safe lead intake from all sources, 1·15 mg. as the maximum permissible and 1·50 mg. as a dangerous intake. Admittedly this estimate is based on a number of assumptions and approximations, but if it is sound it indicates that a total lead intake of roughly 1 mg. daily is the maximum permissible.

Some authorities consider that a total intake of as much as 1 mg. daily may eventually be harmful. They stress the part played by unsuspected lead poisoning, due to the cumulative effect of very small doses, in causing anomalous nervous symptoms and conditions of slight ill health which it is difficult to ascribe to any particular cause. If we take these views into account we must conclude that a daily intake of 1 mg. is to be regarded with suspicion.

On the other hand, Kehoe (1940) has recorded that one of two human subjects ingested 2 mg. of soluble lead daily for six months and the other 1 mg. of soluble lead daily for thirty months, without any symptoms of plumbism even of the vague types, and did not show any blood changes. These may, of course, have been cases of habituation to lead, similar to those recorded for lead workers by Legge and Goadby (1912) and other authorities. There seems to be little doubt that a high degree of tolerance to lead may be established and also that men can stand much more lead than women, according to Legge about three times as much. Another factor already discussed, which is probably of the greatest significance, is the presence of solid food in the alimentary tract, and whether the food contains substances which can combine with and immobilise lead. If, as seems probable, lead in solid food is partly insoluble or non-dialysable, considerably more than 1 mg. daily might be ingested without harm. If Kent's findings referred to above are applicable to the general run of wheat flour, they would suggest that a figure of the order of 1 p.p.m. in some vegetable foods is to be regarded as normal rather than excessive.

With some toxic substances there is the so-called ‘ synergistic ’ action to be considered, where two different substances acting together exert a greater toxic effect than the sum of the two when acting separately. It is possible that such synergistic action may occur to some extent with toxic metals in food, e.g. with lead and zinc, but there is no direct evidence on this point.

In the absence of statutory limits for lead in food the immediate problem is what amount should be regarded by the analyst as warranting the condemnation of a food. It is probably safe to assume that lead in liquids such as beer and cider is absorbed as readily as from water, and certainly no higher allowances should be countenanced for liquids than the limits accepted by most authorities for drinking-water, namely, 0·3 p.p.m. safe, 0·5 the maximum allowable and 0·7 p.p.m. excessive. It is even arguable that for beer these figures should be substantially reduced in view of the large quantities sometimes consumed, up to 1 gallon or more daily, and the possibility of excluding lead altogether from brewery plant. For solid foods the question of limits is more difficult. Kehoe and others (1933, 1940) have given lists of foods with their lead content, and a similar list is given in my report to the Ministry of Health on Lead in Food (1938), from which it appears that the greater number of foods contain either no lead at all or less than 1 part per million. A certain number contain normally between 1 and 2 p.p.m., especially foods which have been dried or concentrated, such as gelatin, meat extracts, spices, dried fruits and vegetables, chocolate, etc. Those which contain still more lead are usually found to have been exposed to some avoidable source of contamination. There should be no insuperable difficulty in producing any food with a lead content of not more than 2 or at most 3 p.p.m. with the exception of shell-fish and crustacea and perhaps synthetic food colours. For a few foods such as spices, tea, meat extracts and phosphate baking powders a higher figure, say 5 or even 10 p.p.m., may be allowable, but consideration should be given to the possibility of producing the food in question without lead, and to the relative amount of the food consumed and its contribution to the total lead intake.

In view of the cumulative action of repeated small doses of lead it is impossible to give any limit below which lead in food can be stated with certainty to be harmless. It should be kept out of food as far as possible. Arsenic in food in amounts over 1 or 2 p.p.m. is now of comparatively rare occurrence ; the same should be true of lead, and any provisional limits adopted by analysts may well be made progressively more stringent wherever it is found that improvement is possible.

THE DETERMINATION OF LEAD IN FOOD

The determination of small amounts of lead in food, from 0·2 to 1 p.p.m., is a more difficult matter than that of similar amounts of arsenic. With arsenic we have, in the Marsh and Gutzeit methods (pp. 189, 191), tests which are practically specific, but with lead interfering metals must be removed or immobilised before any colorimetric methods can be applied. With lead it is harder than with arsenic to secure purity of reagents and to prevent contamination from outside sources during the progress of the analysis.

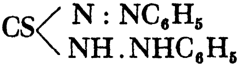
DESTRUCTION OF ORGANIC MATTER. In the A.O.A.C. handbook (1945) preference is given to direct ashing in a muffle furnace at a temperature not above 500°, and this method, with addition of phosphate to prevent loss of lead, is also favoured by Tompsett and Anderson (1935). Usually, however, organic matter is destroyed by wet oxidation with sulphuric and nitric acids or with nitrosyl-sulphuric acid (Francis, Harvey and Buchan 1929). Some analysts use 60 per cent perchloric acid instead of sulphuric, in conjunction with nitric or hydrochloric acid or hydrogen peroxide. Perchloric acid has the advantage that it can more easily be driven off by heat in an open dish on completion of the operation, thus avoiding the use of a large amount of neutralising agent with the attendant risk of introducing lead. The

Association of Official Agricultural Chemists (Official Methods 1940) recommends a mixture of perchloric, sulphuric and nitric acids, which is safe to use and which has an oxidising action much superior to that of the usual sulphuric and nitric acid mixture. A similar mixture is used by Strafford and others (1945).

When nitric acid is used it occasionally happens that a slight yellow tinge remains in the oxidised liquid, which is difficult to remove and which in some processes may interfere with the final colorimetric determination. This yellow colour appears to be caused by the action of nitric acid on fats with the production of substances which are extremely resistant to further oxidation even on prolonged heating with concentrated sulphuric acid. Complete oxidation can usually be effected with perchloric acid. The yellow colour can sometimes be removed by heating the acid residue with about 25 ml. of a saturated solution of ammonium oxalate.

SEPARATION OF LEAD FROM OTHER METALS. Before lead can be determined by colorimetric methods it must be separated from interfering metals, especially iron, and from calcium phosphate, etc. This separation can be effected in several ways, of which the two most important are precipitation as sulphide and extraction with diphenyl-thiocarbazone (' dithizone ').

Separation as Sulphide. Lead may be separated from iron, although not completely, by sulphide precipitation in acid solution. The acidity of the solution should lie between pH 2·5 and 4·5, i.e. within the range of colour change of brom-phenol blue. At higher acidities there is a slight loss of lead owing to solubility of the sulphide, especially in presence of alkaline and alkaline earth chlorides, and at lower acidities iron sulphide may be precipitated with lead (cf. p. 41). When only traces of lead are present a small quantity of a copper salt may be added to assist in precipitating the lead, the two being afterwards separated.

By Extraction with Diphenyl-thiocarbazone (' Dithizone '). This reagent was introduced by Fischer in 1929 and has been very widely used for the determination of traces of heavy metals in food and biological materials. It is a black crystalline powder of formula  $\mathrm{CS}\left\langle\begin{array}{l}\mathrm{N:NC_6H_5}\\ \mathrm{NH.NHC_6H_5}\end{array}\right.$ which dissolves in chloroform or carbon tetrachloride, giving a deep green solution. It is insoluble in water or dilute mineral acids, but readily soluble in dilute ammonia solution. Commercial dithizone may contain oxidation products soluble in chloroform but insoluble in aqueous ammonia. It can be purified by solution in chloroform, extraction with ammonia solution and re-extraction with chloroform after acidification (cf. p. 48).

If the green solution in chloroform is shaken with a neutral or faintly ammoniacal solution containing lead, the lead combines with dithizone forming a compound soluble in chloroform, containing one atom of lead combined with two molecules of dithizone. If sufficient excess of dithizone is present, the smallest traces of lead are completely extracted. The lead complex is red, and the dithizone solution therefore turns from green to red or to an intermediate tint depending upon the relative amounts of lead and dithizone present. Extraction is repeated with fresh quantities of dithizone in chloroform until the green colour remains unaltered, showing that all lead has been extracted.

The reaction is not specific for lead. Practically all heavy metals except the tervalent ones form compounds with dithizone ranging in colour from brown to orange and red in chloroform solution. Zinc in particular gives a bright cherry-red colour similar to that given by lead. On acidification the metallic compounds are decomposed, except those of copper, mercury and silver, and the metal returns to the aqueous solution, the dithizone-chloroform solution again becoming green.

Sylvester and Hughes (1936) find that from a solution buffered with ammonium acetate at about pH 4·5, zinc, copper, mercury, silver, bismuth and cadmium can be extracted with a solution of dithizone in chloroform (p. 124). Lead does not combine with dithizone at pH 4·5 and for its extraction the solution must be neutral or slightly alkaline. In presence of cyanide only lead, thallium, bismuth and stannous tin form complexes with dithizone, so that a separation of lead from copper and zinc can also be effected in this way. For most purposes in food analysis everything extracted with dithizone after wet oxidation and in presence of excess of cyanide in neutral or slightly ammoniacal solution may be considered to be lead. Stannous tin can hardly be present in a solution obtained by wet oxidation. Wichmann and Clifford (1935) suggest that stannic tin may be slightly reduced by dithizone and the resulting stannous tin interfere with the determination, but there is no evidence that this occurs to any appreciable extent. Bismuth may occasionally be present in traces (p. 444). It is retained by cyanide if a large excess of this is present; otherwise it is partially extracted by dithizone. It can be removed from dithizone solution by repeated extraction with aqueous cyanide (Tompsett and Anderson 1935). Willoughby and others (1935) find that bismuth is extracted by dithizone at acidities of about pH 2·0 and can thus be separated from lead. Lead can be separated completely from bismuth by precipitation as sulphate (p. 90). Alternatively the residue after determination of lead may be tested for bismuth by one of the colour tests given on pages 447 and 451. Cadmium may also be extracted by dithizone if insufficient cyanide is present, and appears to require an excess of cyanide for its retention. A tabular summary showing the conditions under which different metals react with dithizone is given on page 131.

The dithizone extract may either be used for the direct determination of lead by comparison of the colour with standards (p. 91) or chloroform may be removed by evaporation, the residue oxidised with a small quantity of sulphuric and nitric acids and lead determined colorimetrically as sulphide (p. 93).

For extraction with dithizone it is necessary to have a clear solution. Colloidal ferric hydroxide or alkaline earth phosphates may interfere with the extraction, but they can usually be kept in solution with ammonium citrate. When much calcium sulphate is present a special procedure is necessary (see below).

Separation of Lead from Large Amounts of Calcium Phosphate. Calcium phosphate, since it is precipitated in neutral or alkaline solutions, interferes with the extraction of lead by dithizone and also with its colorimetric determination as sulphide. Seeker and Clayton (1915), in determining lead in phosphate baking powders by precipitation with hydrogen sulphide, added a large excess of ammonium citrate which keeps the phosphate in solution. Nicholls (1931) has given a somewhat similar method for lead in calcium phosphate, and Kent-Jones and Herd (1933) use this method in conjunction with dithizone extraction.

When materials containing much calcium phosphate are submitted to wet oxidation with sulphuric acid the residue of calcium sulphate may retain lead sulphate. Roche Lynch, Slater and Osler (1934) use a method suggested by Dyer for bringing this calcium sulphate into solution. It depends upon a double decomposition between calcium sulphate and potassium carbonate. The calcium sulphate is separated by filtration and heated with a solution of potassium carbonate for about four hours. This treatment converts it into calcium carbonate with formation of a corresponding amount of potassium sulphate in solution. The calcium carbonate is filtered off and dissolved in acetic acid. The two solutions after neutralisation with ammonia are successively extracted with dithizone and the extract added to that obtained from the main solution.

Lead may also be separated from a large amount of calcium phosphate by dissolving it in acetic acid and adding a solution of potassium oxalate. The lead is carried down by the precipitate of calcium oxalate and can be determined by the usual methods (cf. p. 91). There is, however, always some uncertainty whether the whole of the lead has been precipitated by the oxalate treatment.

Separation with Sodium Diethyl-dithiocarbamate. The use of this reagent for the colorimetric determination of copper is discussed on page 49. It has also been used by Tompsett and Anderson (1935) to separate lead from iron and alkaline earth phosphates before colorimetric determination with dithizone. An extract of the ash of the sample is treated with citric acid, made slightly alkaline, a solution of sodium diethyl-dithiocarbamate added and the compound of lead and diethyl-dithiocarbamic acid extracted with ether. The residue, after evaporation of the ether, is oxidised with sulphuric and perchloric acids and lead determined with dithizone.

Sodium diethyl-dithiocarbamate is not a specific reagent for the separation of lead since it also reacts with copper, zinc and other metals, but these if present are afterwards separated from lead by extraction with dithizone and repeated treatment of the extract with cyanide solution.

Separation by Electrolysis. Lead is deposited as metal on the cathode from solutions of the acetate or complex oxalate and from solutions of the hydroxide in caustic alkali or of the phosphate in alkali or in phosphoric acid solution. From nitric acid solutions, on the other hand, it is deposited as lead peroxide on the anode, lead behaving as if it were in the form of plumbic acid. The disadvantages of separation of metallic lead on the cathode are that the deposit is readily oxidised, and also that it is difficult to prevent a small amount of lead being deposited simultaneously as peroxide on the anode. Cooksey and Walton (1929) determine lead in urine by direct electrolysis after acidification with acetic acid, using platinum electrodes and a current of 0·3 to 0·4 ampere. The lead deposited on the cathode is dissolved off with nitric acid, the nitric acid removed by evaporation and lead determined nephelometrically as sulphite (p. 95). For anodic separation as peroxide the solution, containing 0·01 to 1 mg. of lead in 1 per cent nitric acid, is electrolysed with a low current density using a rotating anode, both electrodes being of platinum. The lead peroxide which separates on the anode may be dissolved off and the lead determined by any of the usual colorimetric methods.

Electrolysis may give low results in presence of phosphoric and sulphuric acids and is inaccurate if the solution contains much iron. Manganese, if present, is likely to be precipitated as dioxide on the anode. It has been stated by various analysts that chlorides, phosphates, nitrites, arsenic, selenium, tellurium, mercury or bismuth may prevent the complete deposition of lead, and bismuth, tin, antimony, manganese and silicon may contaminate the deposit.

The electrolytic method was used by Francis, Harvey and Buchan (1929) to separate lead from copper after precipitation of the mixed sulphides. Brock (1941) gives an account of some recent developments in electrolytic analysis apparatus with special reference to the determination of lead.

If lead peroxide is dissolved from the anode with a solution of sodium acetate and acetic acid, it can be determined colorimetrically with tetramethyl-diamino-diphenylmethane, with which it gives an intense blue colour. The colour is not specific for lead, being given also by manganese dioxide and other oxidising agents.

Lead may be oxidised to peroxide with sodium hypochlorite instead of by electrolysis and determined colorimetrically as above (Klostermann 1926, Seiser, Necke and Müller 1928).

By Precipitation as Sulphate. The separation of lead from other metals by precipitation as sulphate in dilute alcohol is applicable only if the solution is fairly free from iron, otherwise the lead precipitate is liable to be contaminated with ferric sulphate. Calcium sulphate and silica may also contaminate the precipitate. When lead has been partially freed from other metals by precipitation as sulphide or extraction with dithizone, sulphate precipitation serves to separate it from any bismuth which may be present, and also serves to remove entirely the yellow oxidation products resulting from the action of nitric acid on fats. A disadvantage is the slight loss due to the solubility of lead sulphate, a factor which seems to be responsible for the rejection of this method by the Association of Official Agricultural Chemists (1940). This loss can be reduced to 0·005–0·0075 mg. in 15 ml. of aqueous alcohol containing sulphuric acid. In practice a blank test carried through on apparatus and materials almost always shows a positive result, and it must be presumed therefore that the solubility of lead sulphate affects both the test and the blank to an equal extent and can be neglected.

As small a quantity as 0·02 mg. of lead in 15 ml. of alcoholic sulphuric acid gives a distinctly visible precipitate after standing for twelve hours. Smaller quantities cannot be seen as a precipitate, but can nevertheless be separated by filtration and dissolved from the filter-paper with ammonium acetate for colorimetric determination (p. 93).

By Precipitation as Oxalate. Lead may be separated from liquids by precipitation as oxalate in presence of excess of calcium oxalate. Thus Berg and Schmechel (1935) add ammonium oxalate and a soluble calcium salt to wine, filter off the precipitated oxalates and ignite them to carbonates. The carbonates are dissolved in acetic acid and lead extracted with dithizone. The method was originally worked out by Taylor (1928) for lead in urine. There is no evidence in this method that the filtrate from the oxalate precipitation is absolutely free from lead.

Separation of Iron and Bismuth as Thiocyanates. In Hamence's method (1934) organic matter is first destroyed by wet oxidation, and iron, lead and bismuth are precipitated together as sulphides. The sulphides are then brought into solution with nitric acid and the solution treated with pyridine and ammonium thiocyanate. On extraction with a mixture of ether and amyl alcohol, iron is removed as thiocyanate and bismuth as a double pyridine-bismuth thiocyanate. The lead remaining in the aqueous solution can then be determined by the usual methods.

COLORIMETRIC DETERMINATION WITH DIPHENYL-THIOCARBAZONE (DITHIZONE). The use of dithizone for separating lead from other metals has been discussed above. It can also be used for the colorimetric determination of lead. This application of dithizone has been developed particularly by the Association of Official Agricultural Chemists in the United States. The determination may be carried out in various ways.

(*a*) Lead may be extracted from neutral or weakly ammoniacal solution with an excess of dithizone in chloroform in presence of cyanide and the chloroform again washed with alkaline cyanide which removes uncombined dithizone, leaving only the red lead-dithizone compound in solution. This solution is then compared with standards. Tompsett and Anderson (1935) give the colorimetric range as 0·005 to 0·07 mg. in 10 ml. of chloroform or carbon tetrachloride, the best depths of colour for comparison being obtained with 0·01 to 0·02 mg. of lead. As an alternative the lead-dithizone compound may be decomposed, and the lead removed from the chloroform, by shaking with dilute hydrochloric acid, and the resulting green solution of dithizone in chloroform compared with standards (Ross and Lucas 1935, Cheftel and Pigeaud 1936).

(*b*) Lead may be extracted with a slight excess of dithizone, so that the colour of the chloroform extract is a mixture of green and red, the tint varying with the amount of lead present. The colour may be compared with standards or, as in the A.O.A.C. method, measured in a spectro-photometer using light of about 570 mμ.

(*c*) *Titrimetric Extraction Methods*

(i) The lead solution is shaken with successive small measured amounts of a standardised dithizone solution until the latter no longer changes colour from green to red, indicating that all the lead has been extracted. The amount of lead present is then calculated from the amount of dithizone used (Wilkins and others 1935).

(ii) The lead solution is shaken with chloroform to which successive small amounts of a solution of dithizone in chloroform are added until an intermediate tint is reached. To a similar amount of dithizone in chloroform in a second vessel standard lead solution is added with shaking until the tints in the two vessels match (Horwitt and Cowgill 1937, Fischer and Leopoldi 1940). A method of determining zinc and lead in food in this way is given on page 128.

The reaction between lead and dithizone is a reversible one, and the state of equilibrium varies with the pH of the solution as well as with the relative concentrations of lead and dithizone. Lead dithizone itself, without excess of lead salt or of dithizone, is not stable unless the aqueous solution has a pH above 10. For Wilkins' titrimetric method the optimum pH is probably about 9·5. The dependence of the reaction upon the pH of the solution makes it necessary to work within closely defined limits of alkalinity, and to adhere strictly to the conditions for which the methods have been worked out.

For details of the various colorimetric methods using dithizone and a comprehensive discussion of the whole subject the reader is referred to an article by Clifford and Wichmann in the *Journal of the Association of Official Agricultural Chemists* (1936). The subject is also fully discussed by Fischer and Leopoldi (1940).

COLORIMETRIC DETERMINATION AS SULPHIDE. The determination of lead as sulphide appears to have been largely displaced by methods depending on the use of dithizone, but many analysts still prefer it as being more certain in operation and less likely to be affected by small divergences from the prescribed technique. With foods which do not contain much iron, copper or aluminium the colorimetric determination may be carried out on the solution obtained by wet oxidation and neutralisation, without previous separation of lead. The reaction is far more delicate in ammoniacal than in acid solution. Wilkie in 1909 showed that if any iron present is reduced by heating with thiosulphate, and a cyanide added, lead may be determined colorimetrically as sulphide in a slightly ammoniacal solution in presence of iron and copper. Ferrous iron is converted into ferrocyanide, which is colourless in very dilute solution. Sodium thiosulphate is particularly useful as a reducing agent for ferric iron in presence of citric and tartaric acids.

In Francis, Harvey and Buchan's method (1929) lead is separated from all interfering metals by sulphide precipitation followed by electrolysis and precipitation as sulphate. It is then determined colorimetrically as sulphide. Alternatively lead may be determined as sulphide after separation from other metals with dithizone. A general method based on this principle is given on page 99.

0·002 mg. of lead can be detected as sulphide in a 50-ml. Nessler cylinder, i.e. 0·04 p.p.m. in solution, and 0·05 mg.

in 50 ml. is the most suitable quantity for colour matching. A report of a sub-committee of the Analytical Methods Committee of the Society of Public Analysts (1935) discusses the best conditions for the determination, and the degree of accuracy obtainable in ordinary work. Errors of observation can be greatly reduced by using a photo-electric cell for measuring the depth of colour (Frear and Hodgkiss 1936).

Blank Determinations. Under ordinary conditions of work with the method described on page 99 it is not easy to obtain a 'blank' lower than 0·005 mg., which on a 10-g. sample represents 0·5 p.p.m. of lead. If a larger sample is taken wet oxidation takes longer and is more troublesome, more reagents are used and the blank is likely to be higher, so that it is doubtful whether anything is gained thereby, except where liquids are concerned. It is obvious that when determining amounts of lead of the order of 0·2 to 1 p.p.m. a blank of 0·5 p.p.m. is somewhat of a disadvantage, and calls for most accurate and careful work at all stages.

In Francis, Harvey and Buchan's investigations for the Departmental Committee on Ethyl Petrol in 1929 it was found impossible to obtain satisfactory results under the conditions prevailing in a general laboratory. The work had to be carried out in a laboratory set apart for the purpose, and special precautions taken to reduce as far as possible the contamination of apparatus and reagents with traces of lead. Under these conditions the average blank was 0·004 mg. When examining urine for lead it was possible to work on large samples, so that the effect of the blank was minimised and they were able to determine as little as 0·003 to 0·005 p.p.m. of lead in urine.

Clifford and Wichmann (1936) using the dithizone colorimetric method in conjunction with dry ashing of the sample claim to have been able to reduce the blank to 0·001 mg. of lead, and Bambach (1939) even to 0·0001 mg. It may be conceded that the use of sulphuric acid for wet oxidation may introduce traces of lead unless the sulphuric acid is very carefully purified and that dry ashing at a temperature not above 500°, if it can be carried out without loss of lead, which is doubtful, may possibly give a lower blank. The fact remains, however, that Francis, Harvey and Buchan using nitrosylsulphuric acid prepared from sulphur dioxide, and with every possible precaution including the use throughout of silica vessels, could not reduce the blank below 0·004 mg. The term ' blank ' must be understood as meaning not merely a determination of lead in the reagents used, but a complete operation carried through from start to finish in a way precisely similar to the actual test. Nothing short of this will enable accurate determinations to be made of lead in food in amounts approximating to 1 p.p.m.

DETERMINATION AS CHROMATE. Lead chromate precipitated from dilute solutions may be determined iodimetrically, or colorimetrically after solution in nitric acid (Evans 1928). Alternatively chromium in the precipitate may be determined colorimetrically with diphenylcarbazide (Fairhall 1924, Jones 1930). Lead chromate is soluble in water to the extent of about 0·2 p.p.m., but in acetic acid and ammonium acetate solution the solubility is much higher. Huybrechts and Degard (1933) found that the solubility varied from 1·3 p.p.m. in N/40 to 6·2 p.p.m. in N/2 acetic acid, while in N/2 ammonium acetate solution it was as much as 9·4 p.p.m. The solubility varied considerably with temperature. Höll (1935) gives 1·3 p.p.m. as the limiting concentration of lead which can be precipitated as chromate in presence of 5 per cent or less of acetic acid or ammonium acetate. This compares unfavourably with the solubility of lead sulphate in alcoholic sulphuric acid solution which is only 0·3 to 0·5 p.p.m., and is sufficiently high to make the chromate method unsuitable for the determination of very small quantities of lead.

NEPHELOMETRIC DETERMINATION AS SULPHITE. Solutions containing 0·05 p.p.m. of lead give an opalescence with sodium sulphite after standing for some minutes, and this reaction has been used for the determination of small amounts of lead. The reaction is about as sensitive as the sulphide test, over which it possesses no particular advantages. It was used by Cooksey and Walton (1929) and by Taylor (1928) for the determination of lead in urine after separation by electrolysis or by precipitation as oxalate.

SPECTROGRAPHIC METHODS. Spectrographic methods have been applied by several investigators to the determination of traces of lead in foods and biological material, but hitherto it has been questionable whether they have offered any particular advantage over chemical methods. Weyrauch remarked that spectrographic analysis is seldom a short cut but is usually an alternative last stage in a more or less elaborate chemical separation of lead. This is to some extent true of Lampitt and Rooke's method (1933) in which they separate lead sulphate by a modified form of Francis, Harvey and Buchan's method and examine the sulphate in a spark discharge, comparing the intensity of the lead lines 405·8 and 368·3 mμ with standards. Wood (1930) deposits lead electrolytically on a gold cathode and examines the deposit in a spark discharge. Harper and Strafford (1942) have also described a spectrographic method for lead, arsenic and copper in food colours in which they use cadmium as an internal standard. The material is first subjected to wet oxidation and the metals precipitated as sulphides. The sulphide precipitate is collected on a small filter pad of powdered graphite and examined in a direct-current low-tension arc between graphite electrodes. The intensities of the lines on the photographic plate are compared visually.

In these methods more or less preliminary chemical separation is involved, but in others the ash of the food is examined direct in the arc or spark discharge. Thus Blumberg and Scott (1935) determine lead directly in the ash by photographing the carbon arc spectrum and comparing the photograph with those given by a similar material to which known amounts of lead have been added. The lead lines used in making the comparison are those of wavelength 261·4, 280·2, 283·3, 287·3, 357·3, 363·9, 368·3, 374·0 and 405·8 mμ, of which 283·3 and 405·8 are the most persistent and 357·3 and 374·0 the weakest. Cholak (1935) adds a known amount of bismuth to the material and uses it as an internal standard, the intensity of the bismuth line 289·8 being compared with that of the strongest lead line 283·3 mμ. Tracy and McPheat (1943) find that bismuth is not a trustworthy internal standard for blood ash, since blood may contain traces of bismuth of therapeutic origin ; they prefer to use platinum which gives a spectral line at 283 mμ close to the lead line 283·3.

These methods, in so far as they depend on visual comparison of the intensity of the spectral lines, are only approximately quantitative, but have the advantage that they afford specific proof of the presence of lead and are therefore valuable in confirming the results of chemical analysis. Kent (1942) uses a method in which the comparison of the relative intensity of the lines is made with a micro-photometer which makes it possible to obtain more consistent results. The material is ashed and the ash treated with a known amount of a standard bismuth solution. A few milligrams of the treated ash are volatilised in a low-voltage copper arc with a current of 2 amperes. The arc light is photographed through a spectrometer with an exposure of 60 seconds. The photographs are compared with those obtained in a precisely similar way from a standard ash containing known amounts of lead together with varying amounts of bismuth. The relative intensities of the lead and bismuth lines on the negatives are compared by means of a micro-photometer. By using suitable sources of light (oxy-coal-gas flame, low-voltage direct-current arc or high-voltage alternating-current spark) with magnesium or iron as internal standards, the method has been applied to the determination of lithium, boron, vanadium, gold, silver, manganese, nickel, cobalt and tin in foods and biological material. In some instances, e.g. lead and tin naturally occurring in cereal products, Kent's method seems to give considerably higher figures than those obtained by chemical methods (pp. 81, 144), and it is not clear whether the direct-current arc is in fact capable of giving accurate and reproducible results. Kingsbury and McClelland (1945) consider that the two common methods of excitation used in spectrographic analysis, the direct-current arc and the high-voltage condensed spark, are not, as a general rule, suitable for accurate quantitative trace analysis. The arc is sensitive but not regular enough in operation, and the spark is not sufficiently sensitive. They use an intermittent alternating-current arc which, they find, combines the sensitivity of the arc with the reproducibility of the spark. The voltage is 200 to 250, and the current 4 amperes, the frequency of the alternating discharges being 100 per second. Iron is used as an internal control, and the relative intensities of the spectral lines on the photographic plate are compared by means of a microphotometer. According to McClelland (1946) the limits within which metals can be determined are roughly 1 to 25 μg. per millilitre, with a degree of accuracy of about ±5 per cent.

POLAROGRAPHIC METHOD. The principles of this method have been outlined on page 53. Cholak and Bambach (1941) apply it to lead in biological material by ashing the sample at 500° and separating the lead by electrolysis. This is dissolved from the electrode and concentrated into a small volume of a solution containing tartaric and nitric acids and a known amount of cadmium. A polarograph of the solution is taken and the amount of lead found by comparison of the inflexions of the voltage-current curve caused by cadmium and lead respectively. The method is stated to be as accurate and sensitive as the dithizone method.

The direct determination of lead without previous separation from other metals can be effected only if the ‘ wave ’ in the voltage-current curve due to lead can be separated from those due to ferric iron and stannous tin, which coincide or interfere with it. Jones and Brasher (1947) find that if ferric iron is reduced with hydroxylamine and the pH of the solution is adjusted to between 2·5 and 3·0, interference by these two metals can be eliminated.

NITRITE TEST FOR LEAD. Lead can be detected microchemically in tissues by means of the triple nitrite of potassium, lead and copper. The lead is first precipitated together with copper as sulphide, the precipitate dissolved in nitric acid and the solution evaporated on a microscope slide.

The residue is treated with a drop of a solution of acetic acid, ammonium acetate and potassium nitrite. The presence of lead is shown by the formation of black cubical crystals of the triple nitrite.

GENERAL METHOD FOR DETERMINING LEAD IN FOOD AND BIOLOGICAL MATERIAL. The various analytical processes discussed above can of course be combined in different ways. Perhaps the best method for food and biological material is the one given by Allport and Skrimshire (1932). The stages of the method are (i) wet oxidation of organic matter, (ii) extraction with dithizone, (iii) colorimetric determination as sulphide. The method does not differentiate between lead and bismuth, but bismuth is rarely present in foods (p. 444), and for most purposes may be disregarded. If it is desired to exclude the possibility of any bismuth being returned as lead, dithizone extraction may be followed by sulphate precipitation. This also has the advantage, with fatty foods, that it completely removes the persistent yellow tinge which sometimes interferes with the colorimetric determination. The following method, which has been found by me to give very satisfactory results, is a combination of those given by Allport and Skrimshire (1932) and by Francis, Harvey and Buchan (1929).

A suitable quantity of the sample, usually 5 g. but varying with the nature of the material and the amount of lead present, is oxidised with 5 ml. of sulphuric acid and the least possible quantity of nitric acid. The residue is diluted with water and again concentrated until white fumes are evolved, in order to remove as much as possible of the nitric acid. It is again diluted with about 20 ml. of water, 2 g. of citric acid added and the solution boiled and cooled. With most foods the solution at this stage is clear or contains only a slight residue of silica which does not interfere with subsequent dithizone extraction. If much calcium sulphate separates it must be filtered off and treated with potassium carbonate as described later. If only a small amount of insoluble matter is present the liquid is filtered through a 7-cm. filter (preferably Whatman No. 44) which has been previously washed with hot diluted hydrochloric acid (1 : 1), and the residue on the filter is thoroughly washed with 10 ml. of hydrochloric acid of this strength and finally with hot water. The clear solution is concentrated to about 50 ml. and cooled. It is neutralised to litmus paper with 0·880 ammonia solution and 0·5 ml. of ammonia is added in excess. The ammoniacal solution should now be extracted with dithizone without delay before it has had time to become cloudy from separation of phosphates, etc. For this it is cooled quickly to 30°, 1 ml. of a 10 per cent potassium cyanide solution is added, and the liquid transferred to a 250-ml. separating funnel. The volume with washings should be about 100 to 150 ml. It is extracted with three successive portions of a 0·1 per cent solution of dithizone in chloroform. 10 ml. of the reagent solution should be used for the first extraction and 5 ml. for the second and third. It may be necessary to continue the extraction with further portions of reagent until no colour change from green to red takes place. The liquid should be shaken vigorously for about one minute for each extraction. Each extract is washed in turn with about 10 ml. of water in a small separator, the chloroform extracts combined and transferred to a 6″ × 1″ Pyrex test-tube, and the solvent evaporated off in a hot-water bath.

The residue from the chloroform is now heated with 0·7 ml. of sulphuric acid and a few drops of nitric acid until all organic matter is destroyed. The residual acid is diluted with water and reconcentrated until it fumes freely. The tube now contains the whole of the lead originally present, in about 0·5 ml. of sulphuric acid. 15 ml. of a mixture containing one volume of alcohol and two volumes of water are added and thoroughly mixed with the acid. The mixture is allowed to stand overnight and, whether any precipitate is visible or not, filtered through a 5-cm. close-grained filter-paper (Whatman No. 44) which has been washed with hot diluted hydrochloric acid (1 : 1) and hot water just before use. The filter is then washed twice with a few millilitres of a mixture of alcohol, water and sulphuric acid in the respective proportions by volume of 10, 20 and 1. The filtrate and washings are rejected. 10 ml. of 10 per cent ammonium acetate solution are now boiled in the tube in which the sulphate precipitation was carried out and the hot solution is passed through the filter. This operation is repeated, the same 10 ml. of ammonium acetate solution being again raised to boiling-point and passed through the filter. The filter is finally washed three times with about 5 ml. of hot water containing a little ammonium acetate.

A rough estimate of the quantity of lead present having been formed from the appearance of the sulphate precipitate, the whole or a suitable part of the combined filtrate is transferred to a 50-ml. Nessler cylinder of colourless glass. A similar cylinder is used for the comparison solution and 10 ml. of 10 per cent ammonium acetate solution are placed in this cylinder. To each are added 1·5 ml. of 0·880 ammonia, 1 ml. of 10 per cent potassium cyanide solution, water to the 50-ml. mark and finally 2 drops of a colourless freshly prepared 10 per cent solution of sodium sulphide, and the contents mixed. A newly made lead solution, containing 0·01 mg. of lead per millilitre, is added from a burette to the control until the tints match. The control is then rejected and a fresh one prepared containing all the reagents except the sulphide. To this is added a volume of the lead solution which is less by 1 ml. than that used for the first comparison. The sulphide is then introduced and standard lead solution added until the tints match. This second matching is unnecessary when the amount of standard lead solution required does not exceed 2 ml. The cylinders should be viewed with the control on the left and also on the right of the test solution.

A blank experiment must be performed on the apparatus and reagents with each set of determinations, being carried through from start to finish in a manner closely similar to that of the actual determination, and the lead so found subtracted from that found in the sample. The amount of lead found in blank experiments is usually about 0·005 mg. and should not exceed 0·01 mg. The utmost care must be taken to prevent contamination with lead from glass-ware, reagents or dust from the air (cf. remarks on blank determinations, p. 94).

Modification for use with Samples containing much Calcium Phosphate. If the sample contains much calcium phosphate the liquid after destruction of organic matter will contain a heavy precipitate of calcium sulphate which may retain lead. In such an event, Roche Lynch's method (1934) may be used as follows.

The acid solution, after dilution, is transferred to a 100-ml. centrifuge tube and the precipitate separated in the centrifuge, the flask being washed out with a little ammonium acetate solution. The clear liquid is decanted back into the original flask, the residue is washed twice in the centrifuge tube with about 20 ml. of hot water and the washings are added to the liquid in the flask. To the residue are added 4 g. of potassium or sodium carbonate and about 100 ml. of water and the vessel placed in a boiling water bath for about four hours, the contents being frequently stirred. The sides of the tube are washed down with water and the mixture cooled and centrifuged. The residue of insoluble carbonate is washed in the centrifuge once with cold water, the clear solution and washings being added to the solution in the original flask. This solution now contains the ' soluble lead ' and phosphate component. It is often coloured by iron salts. 2 to 4 g. of citric acid, according to the amount of iron present, are added and then 0·880 ammonia until an excess of 0·5 ml. is present. 1 ml. of 10 per cent potassium cyanide solution is then added and lead extracted with dithizone as described above.

The residue of calcium carbonate contains the ' insoluble lead '. It is mixed with about 50 ml. of water and acetic or hydrochloric acid is added until the carbonate is dissolved. The solution is boiled to remove carbon dioxide, 2 g. of citric acid added, then 0·880 ammonia until an excess of 0·5 ml. is present, then 1 ml. of 10 per cent potassium cyanide solution. The whole is diluted to about 100 to 150 ml. and lead extracted with dithizone.

The combined dithizone extracts are taken as above described through the sulphate precipitation process and lead determined as sulphide. This method usually gives higher blanks than the one previously described, from 0·012 to 0·015 mg.

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3

[ZINC]

ZINC IN HUMAN AND ANIMAL TISSUES. Zinc exists in the body in greater quantity than copper, but resembles copper in its relative distribution in the various organs. Eggleton (1940) gives figures showing the relative absorption of zinc and of copper by different organs of the body. Zinc is largely concentrated in the liver and spleen. Human adult livers, with 50 to 140 p.p.m. of zinc, contain rather more than those of infants, with 40 to 80 p.p.m. (Rost 1921). Mammalian livers, other than human, contain in general from 20 to 40 p.p.m. (McHargue 1925, Bertrand and Vladesco 1922) and sheep livers considerably more (Ivanov 1938). In white rats the liver at birth contains about 200 p.p.m. in the fresh tissue, but this decreases during growth, becoming 100 p.p.m. at 15 days and 20 to 60 p.p.m. in the adult animals (Bertrand and Brandt-Beauzemont 1930, Heller and Burke 1927).

Zinc is the latest of the trace elements to be shown to have a definite function in the body. It is present in mammalian blood to the extent of about 6 to 7 p.p.m., and Keilin and Mann (1940) have shown that it forms the prosthetic group of the enzyme carbonic anhydrase. This enzyme, discovered by Meldrum and Roughton in 1933, contains 0·33 per cent of zinc, the protein in the molecule being combined with two atoms of metal. Red blood corpuscles contain about 0·2 per cent of carbonic anhydrase. This corresponds to 7 p.p.m. of zinc in the corpuscles and accounts for about half of the zinc present in human blood. Carbonic anhydrase is also present in the mucous membrane of the stomach and in the mammalian pancreas. Its function is to catalyse both phases of the reversible reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$. It plays an important part in the transport of carbon dioxide by the blood and its activity is directly or indirectly linked with the reactions in the acid-base equilibrium of the body.

Carbonic anhydrase is also concerned in the formation of egg-shells, and disturbance of its function is said to lead to soft-shelled eggs in hens.

Human milk contains 1·3 to 2 p p.m. of zinc, and cows' milk 3·5 to 4 p.p.m. (Rost 1921). The zinc content of cows' colostrum is about three times that of milk (Koga 1934). Broek and Wolff (1935) give 3·5 p.p.m. for human milk, 3·4 p.p.m. for cows' milk and 5·8 p.p.m. for colostrum.

Eggleton (1939) found large amounts of zinc, averaging 200 p.p.m., in human hair and nails, and Cruickshank (1936, 1940) found similar amounts in teeth. Like lead, it tends to accumulate in the bones ; 100 to 200 p.p.m. have been found in human and animal bones (Drinker and others 1927, Grimmett and others 1937).

ZINC AND NUTRITION. Experiments on rats have shown that traces of zinc in food are indispensable. The daily requirement of rats is about 0·04 mg. and is met by food containing about 3 p.p.m. of zinc (Hove and others 1937). If only half of this is given the rats show a fifty per cent decrease in growth, and their hair does not grow properly ; also they develop pellagra-like lesions round the eyes and mouth. The immediate effect of lack of zinc seems to be delay in intestinal absorption. Possibly the carbonic anhydrase of the mucous membrane may be concerned in this, or intestinal phosphatase which is activated by zinc. If nearly all zinc is removed from their food, rats develop keratosis of the oesophagus with a thick layer of partially keratinised cells. The skin also shows keratinisation. The eyes show changes which are also associated with riboflavin deficiency, and Follis and others (1941) suggest that zinc deficiency may impair the absorption or utilisation of riboflavin. The correlation between zinc and aneurin claimed by Eggleton (1939) and referred to below, is interesting in this connection. In complete absence of zinc, reproduction is seriously affected (Newell and McCollum 1933). According to Mussill (1941) zinc deficiency is one of the causes of sterility in heifers. Davidson (1942) suggests that the enzyme uricase, which catalyses the oxidation of uric acid to allantoin, may contain zinc as an essential constituent.

Human requirements of zinc in food are given tentatively by Scoular (1939) as 0·3 mg. per kilogram for a child, which would correspond to 7 mg. daily for a child of about 50 lb. weight. Eggleton (1939), using data from various sources for the zinc content of foods, calculated that the normal daily intake of zinc in food is about 12 mg. No suggestion has been made that zinc deficiency may occur in this country, but metabolism experiments on children in the United States ingesting 15 to 16 mg. of zinc daily in food have shown considerable retention, which is interpreted as indicating fairly high zinc requirements (Scoular 1939, Stern and others 1941). In South China the daily intake of the poorer class of people is only 6 mg. (Eggleton 1939). The foods consumed (sea-fish, cooked polished rice and cabbage) are also poor in aneurin (vitamin B_1), and Eggleton points out that there is a strong positive correlation between the amounts of zinc and of aneurin in foods generally. Sufferers from beri-beri due to lack of vitamin B_1 showed a reduction of zinc in their tissues. Their hair, nails and epidermis contained only half the amount found in healthy persons. He suggests that zinc may be a factor in the beri-beri syndrome.

Zinc leaves the body mainly by way of the faeces. Drinker, Fehnel and Marsh (1927) gave the average daily excretion of an adult as 0·9 mg. in urine and 10 mg. in faeces and showed that a sudden increase in zinc intake had no effect on the urinary excretion. Rost (1921) gives extreme limits of 0·6 to 1·9 mg. daily in urine and 3 to 9 mg. in faeces. McCance and Widdowson (1942) give a much lower normal urinary excretion, 0·3 mg. daily, and confirm Drinker's observation that this is practically unaffected by increased intake. Montgomery and others (1943) using a radioactive isotope find that, in dogs, zinc after absorption is largely eliminated in the pancreatic juice.

ZINC AND INSULIN. Zinc is of value in insulin therapy. In 1936 it was found that the addition of a protamine to insulin gives a protamine-insulin which is less soluble in the body fluids than insulin itself. Absorption is therefore slower, and the complex continues to delay accumulation of sugar in the blood for more than twice as long as insulin alone. Scott and Fisher (1936) found that the addition of very small amounts of zinc still further prolongs the hypoglucaemic action of protamine-insulin. Protamine-zinc-insulin is now used widely for the treatment of diabetes.

Zinc is not alone in its action on insulin. A few other metals, in particular magnesium and iron, have a similar effect. Insulin can be crystallised only in presence of traces of heavy metals, zinc, nickel, cobalt and cadmium, and the resulting crystals show constant values for the respective metals, of the order of 0·5 per cent, indicating that they contain the metals as chemically combined constituents and not as impurities (Scott and Fisher 1935, Fisher and Scott 1938, Sahyun 1941).

According to Scott and Fisher the pancreas of diabetic patients contains only half as much zinc as the normal pancreas. They suggest that zinc may be concerned with insulin storage. Zinc insulinate is not the active form of insulin in metabolism (Krebs and Eggleston 1938). Further work on the combination of zinc and insulin has been carried out by Eisenbrand and others (1942).

ZINC AND PLANT GROWTH

Zinc Requirements of Plants. Small amounts of zinc appear to be necessary to plant growth, but larger quantities are toxic. The stimulating action is particularly pronounced in lower plants and fungi. In common with other metals zinc helps the growth of *Aspergillus niger* (McHargue and Calfee 1931, Steinberg 1934) and appears to be essential to enable this mould to synthesise cellulose or poly-saccharides from mono-saccharides (Kauffman-Cosla and Brüll 1935). Above a certain concentration it is toxic, the optimum concentration being about 8 p.p.m. In 100 species of fungi Mousseron and Fauroux (1932) found from 14 to 279 p.p.m. of zinc in the dry tissue. For yeast growth the optimum concentration is given as 10 p.p.m. (McHargue and Calfee 1931). Other workers have found that 0·001N zinc solutions (32 p.p.m.) have no appreciable effect on fermentation, and 0·002N solutions (65 p.p.m.) inhibit it (Zlataroff and others 1931). Small amounts of zinc stimulate the germination of seeds (Ionesco 1932).

The beneficial action of zinc is less evident in green plants, but small quantities, up to 0·05 p.p.m. in water culture, must always be present. Its activating effect appears to be greatly reduced as soon as the seedling produces green leaves, and chlorophyll can assume the function of synthesising nutrient material for the plant (Gračanin 1928). If, however, the formation of chlorophyll is prevented by exclusion of light, zinc salts continue to act as activators of growth. Possibly the stimulating effect of zinc is connected with the action of an enzyme similar to the carbonic anhydrase of blood corpuscles. The assimilation of carbon dioxide is the main function of chlorophyll, and it may be that the activities of zinc and chlorophyll in this respect are closely connected. Bertrand (1933) has pointed out that the amount of zinc in plants is to some extent correlated with the chlorophyll content and that etiolated leaves contain only half as much zinc as green ones. Thatcher (1934) considered that zinc and copper are mutually concerned with oxidation and reduction processes in plant tissues, and Reed and Dufrenoy (1935) put forward the view that zinc may be a factor, together with sulphydryl compounds, in the regulation of the oxidation-reduction potential. According to Skoog (1940) the role of zinc in plants is connected with the maintenance of auxin, the growth hormone. Reed (1942) finds that zinc is essential for seed production in plants.

Effects of Zinc Deficiency. Fertile soils contain from 1 to 5 p.p.m. of zinc and in exceptional instances much more, (Hibbard 1940). Zinc deficiency in soils is well known. It has been shown to be the cause of the so-called ‘ rosette ’ disease of pecan trees, a nut-producing species of hickory (*Carya olivaeformis*) common in parts of the United States. This disease also attacks other fruit trees, e.g. cherries. Mottle-leaf disease of citrus trees in Florida, ‘ bronzing ’ of tung-oil trees in Louisiana, white bud of maize and chlorosis of many species of plants are stated to be due to lack of available zinc in the soil. Zinc deficiency may occur on basic soils even when the zinc content is considerable, but not usually on acid soils although the total amount of zinc may be much smaller. These diseases can be controlled not only by dressings of zinc sulphate applied to the soil, but also by spraying the plants with zinc sulphate or dusting with zinc oxide.

A possible explanation of the action of zinc in some of these conditions is that they are primarily of bacterial or protozoal origin and that zinc in solution is toxic to the particular organisms concerned (Ark 1937).

Toxicity of Zinc to Plants. A high concentration of zinc, of the order of 60 p.p.m. in a nutrient solution, is toxic to plants, but a great deal depends on the nature of the zinc salts used and the character of the soil. It has been found that while the growth of plants in water culture is adversely affected under certain conditions by as little as 5 p.p.m. of zinc sulphate, plants can grow on highly zinciferous soils and can take up relatively large amounts of the metal, from 0·2 to 1 per cent of the plant tissue, without apparent injury. Zinc tends to accumulate in the surface layers of woodland soils owing to the decay of leaves. Rao (1937) refers to certain soils in India containing as much as 300 to 600 p.p.m. and still fertile. Clearly the greater part of this zinc must be insoluble and relatively inert.

On zinc-deficient soils a dressing of 20 lb. of zinc sulphate per acre will cure chlorosis in maize (Barnette 1934), but heavy dressings of the order of 500 to 700 lb. per acre, corresponding to about 70 to 100 p.p.m. of zinc in the top eight inches of soil, are toxic.

THE ZINC CONTENT OF FOODS. The following is a rough division of some common foods according to the amount of zinc recorded as having been found in them. No differentiation has been made between natural and adventitious zinc.

0·25 to 2 p.p.m.—apples, oranges, lemons, figs, grapes, chestnuts, pulpy fruits generally, etiolated green vegetables, mineral waters, honey (comb).
2 to 8 p.p.m.—raspberries, loganberries, dates, green vegetables, most sea-fish, lean beef, milk, polished rice, beetroot, sugar-beet, bananas, celery, tomatoes, asparagus, carrots, radishes, potatoes, mushrooms, coffee, white flour and bread.
8 to 20 p.p.m.—some cereals, yeast, onions, rice (whole), whole eggs, almonds, potted fish, potted meat.
20 to 50 p.p.m.—wholemeal flour and bread, oatmeal, barleymeal, cocoa, molasses, egg yolk, rabbit, chicken, nuts, peas, beans, lentils, tea, dried yeast, cockles, mussels.
Over 50 p.p.m.—wheat germ (140), wheat bran (75 to 140), oysters (270 to 600), beef liver (30 to 85), gelatin, dried eggs.

Fish. Most sea-fish have a comparatively low zinc content, but Bertrand (1921) gives the extremely high figures of 700 to 1,200 p.p.m. for herrings. He also refers to an amount as high as 7,060 p.p.m. in a tench seven years old. Elsdon (1932) found 100 p.p.m. in canned lobsters which may have been due partly to accidental causes, or may possibly have been intentionally added to remove sulphur as zinc sulphide and prevent blackening of the contents by ferrous sulphide (p. 253). Very high figures have been found for oysters, up to 2,000 p.p.m. Portuguese oysters (*Gryphaea angulata*) are stated by Bertrand to contain 500 to 1,300 p.p.m. of zinc in the fresh material. It would seem that these oysters would be likely to cause vomiting unless the zinc is in a form which is relatively non-irritant to the stomach. Webb (1937), as a result of spectrographic analysis of marine invertebrates, found a great deal of zinc in some species, but none in others closely related. Sea-water, according to Atkins (1936), contains less than 0·008 p.p.m. of zinc, a figure similar to that for copper, and according to Allport and Moon (1939) 0·06 p.p.m. Bertrand (1939) gives a far higher figure, 3 to 4 p.p.m., but his method involves the addition of comparatively large amounts of reagents which may introduce traces of zinc.

Water. The permissible limit for zinc in drinking-water is usually taken as 5 p.p.m. Water containing this amount, if bicarbonates are also present, may turn opalescent, show an iridescent film on boiling and deposit a greyish-white paint-like coating on the surface of the vessel (Howard 1923). Hard water has scarcely any action on zinc if all air is excluded. If the water is fairly free from chlorides, especially if it is a hard water, zinc becomes coated with oxide or basic carbonate and is not further attacked. Chlorides favour the solution of zinc in water, and chlorination followed by conversion of excess of chlorine into chloride may increase the amount taken up to more than 50 p.p.m., but it is stated that if free chlorine is left in the water the amount of zinc taken up is inappreciable (Lothian and Ward 1922). Distilled water dissolves more zinc than hard water, and saturation with carbonic acid greatly increases corrosion (Rincke 1914), so that carbonated water may on occasions contain excessive amounts. Water containing oxygen will dissolve zinc from brass pipes, especially if the water is soft and acid (Haase 1933).

Allport and Moon (1939) found that a sample of London tap water contained only 0·23 p.p.m.

Liquid Foods and Beverages. Milk is specially liable to take up zinc. It is said that milk kept in zinc vessels shows less tendency to become sour, possibly owing to the formation of sparingly soluble zinc lactate, lactic acid being thus removed as it is formed. In common with nickel and copper, the solubility of zinc in milk shows a maximum at 75° to 80° C. Kerr (1942) has shown that milk-bottle caps made of a tin-zinc alloy give up amounts of zinc of the order of

30 to 60 p.p.m. to the cream layer, corresponding roughly to 3 to 6 p.p.m. in the whole milk.

Alcoholic, acid or saline liquids are particularly corrosive to zinc. Thus Schauffele found that the amounts dissolved in 15 days by different liquids from zinc and galvanised iron vessels were as follows.

	G. zinc per litre	
	Zinc vessel	*Galvanised iron vessel*
Brandy	0·95	0·70
Wine	3·95	4·10
Vinegar	31·75	60·75
Weak soup	0·86	1·76
Milk	5·13	7·00
Salt water	1·75	0·40
Fresh water	trace	trace

Sale and Badger's figures (1924) for zinc dissolved in 17 hours from galvanised pails by various drinks are,

	Mg. zinc per litre
Tap water	5
Distilled water	9
Carbonated water	193
Milk	438
Orangeade	530
Lemonade	1411

Zinc acquires a protective coating in brewery worts and beer (Lowy 1935), but enough dissolves to give an unpleasant metallic taste (Matthews 1929).

Wines containing sulphur dioxide may act upon zinc with formation of hydrogen sulphide which then reacts with more sulphur dioxide, precipitating sulphur and causing turbidity. This may occur also with iron, tin and aluminium (Grelot 1922). Bodmer in 1905 referred to the occurrence in Moselle wine of a blue sediment containing zinc which he attributed to a process of fining wine with potassium ferrocyanide and zinc sulphate. Several patents for the fining of wine with zinc ferrocyanide were taken out in Germany about the year 1903, but the process was subsequently forbidden (p. 250). Normally the zinc content of wine is very small, below 5 p.p.m.

Other Foods. Beet molasses and treacle have a high zinc content (15 to 50 p.p.m.), owing partly to concentration of the 2 or 3 p.p.m. natural to sugar-beets and partly to the use of zinc-coated vessels in sugar refineries. Zinc is more efficient than tin as a protection for iron against attack by sugar solutions : tin-plate corrodes readily and colours the sugar (cf. p. 146). There is, therefore, always a possibility of zinc being found in sugar products (Spengler and Zablinsky 1929). Maple sugar often contains relatively large amounts and run honey also, up to 17 p.p.m. (Schuette and Zimmermann 1940). Artificial honey has shown 88 to 305 p.p.m. (Rausching 1942). Confectionery will take it up to the extent of 15 to 30 p.p.m. if wrapped in zinc foil. Chocolate which had been in contact with zinc foil for two weeks showed on analysis 4·6 to 65 p.p.m. (Fairhall and Walker 1929).

Gelatin prepared from hide waste and tannery refuse may contain as much as 0·1 to 0·2 per cent of zinc. This may be due partly to the fact that zinc is concentrated naturally in epidermal tissues and partly to the use of zinc-coated vessels, or to galvanised wire netting on which the gelatin is dried. The United States limit for zinc in gelatin is 100 p.p.m., and a similar limit is in force in this country under the Edible Gelatin (Control) Order, 1947.

Dried fruit may contain zinc if it has been dried on zinc trays. Analyses carried out in Germany in 1896 showed 100 to 500 p.p.m. of zinc oxide. Excessive amounts have also been found in jam made in galvanised iron pans.

Zinc has been used instead of copper for greening canned and bottled peas. Cribb and Still (1925) found up to 440 p.p.m. in several samples which had retained their green colour although no copper was present. They suggested that the magnesium of chlorophyll had been replaced by zinc and that such replacement had the effect of stabilising the colour (cf. Hill 1925). The colouring of food with compounds of zinc is prohibited in this country under the Preservatives Regulations 1925–7. It is a moot point whether this prohibition would extend to zinc salts added for the purpose of preventing discoloration in canned crab, etc. (p. 253).

The Departmental Committee on Preservatives and Colouring Matters in Foods (1924) recommended that artificial colours used in food should not contain more than 20 p.p.m. of lead, copper, tin and zinc together. Seeing that the commonly accepted limit for tin in canned foods is 286 p.p.m. (p. 144) and also that many foods may contain naturally much more than 20 p.p.m. of zinc, it is not clear why such a very stringent limit was recommended for these two metals in artificial colours.

TOXIC ACTION OF ZINC. Moerner (1898) stated that water containing 7 to 8 p.p.m. of zinc had been consumed for years without ill effects despite its bitter taste, and Wolffhügel (1887), as a result of inquiries in 20 towns where galvanised pipes and containers were used for the water supply, could find no evidence of injury to health. Bartow and Weigle (1932) say that certain United States water supplies contain as much as 50 p.p.m. of zinc and no ill effects have been observed in persons drinking such water. It must have had an unpleasant metallic taste. Soda-water containing 57 p.p.m. of zinc has been reported in Australia to have caused vomiting, but the subject may have been unusually susceptible. The lowest emetic dose of zinc sulphate in the British Pharmacopœia is 10 grains, corresponding to about 147 mg. of zinc. It is likely that soft water may dissolve dangerous amounts (p. 114), and several outbreaks of poisoning have been attributed to drinking rain-water collected from galvanised iron roofs or stored in galvanised iron tanks. Some of the earlier recorded instances of this may have been due to unrecognised bacterial food poisoning, but it is stated by competent authorities to have occurred on several occasions among troops in the war of 1914–18.

Excessive quantities of zinc are readily taken up if acid foods are kept, even for a short time, in zinc-coated iron vessels. In 1922 an outbreak of poisoning characterised by violent vomiting happened in an institution near London. This was caused by stewed apples prepared in galvanised iron vessels and containing over 800 p.p.m. of zinc. A similar occurrence took place in 1938 in Holland. Tomlinson (1944) reports an outbreak involving 200 persons which was traced to dried apple rings cooked in galvanised iron vessels. The uncooked rings contained 340 p.p.m. of zinc and the cooked rings 1,410 p.p.m. Cruickshank (1944) gives details of another outbreak involving 35 pupils of a girls' school. A case of poisoning due to drinking bottled root beer containing 229 p.p.m. is reported by Sale and Badger (1924). Cider may take up as much as 1,200 p.p.m. if drawn off into zinc-lined vessels.

Apart from vomiting caused by large doses of zinc in food there are no records of chronic poisoning in man. Prolonged treatment with zinc salts in medicine has been said to cause symptoms like those of lead poisoning, but it must be borne in mind that zinc salts are occasionally contaminated with lead. The inhalation of zinc fumes in industry leads to malaise, irritation of the respiratory tract, catarrh, salivation, thirst and high temperature (Molfino 1937), but zinc in these cases is quickly eliminated from the system and its action appears to be in no way cumulative. Presumably there is a range within which the zinc intake is not high enough for it to act as an emetic but sufficiently high for it to interfere with metabolism. The lower limits of such a range are not known. Experiments on rabbits, animals which do not vomit, have shown that daily doses of 34 mg. of zinc per kilogram of body weight are fatal in 11 days (Jaeger 1931). This would correspond to 770 mg. daily for a child of 50 lb., or five times the emetic dose. In New Zealand in 1936 about 40 or 50 young pigs died after being fed on skim milk passed through galvanised iron pipes. Investigation showed that the milk took up 490 p.p.m. of zinc from the pipes (Grimmett and others 1936, 1937). In subsequent experiments pigs at weaning age were fed for two months on whole milk containing 1,000 p.p.m. of zinc and suffered from lameness and malnutrition with accumulation of zinc in kidneys, liver and bones. The pigs each consumed about 2·5 g. of zinc daily, a very large dose. Lehmann fed a dog for 335 days on food containing zinc carbonate, during which time 155 g. of zinc were taken without any appreciable effect on health, although a post-mortem examination showed zinc in almost all the organs. Drinker and others (1927) gave from 0·175 to 1·0 g. of zinc oxide daily to dogs and cats for periods ranging from 3 to 53 days with no apparent injury. Heller and Burke (1927) found that rats fed on food containing 0·25 per cent (2,500 p.p.m.) of zinc in the form of metal, chloride or carbonate, showed no injury whatever through three generations. There was only slight retention of zinc in the organs. With 0·5 per cent of zinc as oxide there were indications of interference with growth, and with 0·5 per cent as chloride a heavy mortality in the young. Sutton and Nelson (1937) found that 1 per cent of zinc as carbonate in the diet, a relatively enormous dose, impaired the growth and health of rats. In a later paper (1939) Sutton states that food containing 0·5 per cent of zinc in the form of zinc sulphate caused anaemia and death in ten to twelve weeks, while 0·1 per cent had very little effect. Bartow and Weigle (1932) gave rats 1,000 p.p.m. of zinc sulphate in their drinking-water for 40 days without fatal results, although the gain in weight was less than that of controls. Graham and others (1940) gave daily doses of zinc varying from 12 to 120 g. in the form of zinc lactate over a period of 2½ years to breeding mares without any toxic symptoms.

It is difficult, from the results of these experiments, to draw conclusions as to the possibility of chronic zinc poisoning in man or to suggest limits for the amount allowable in food. It is clear that the irritant action of zinc salts on the stomach is largely dependent on the kind of zinc salt ingested and the nature of the food. It would seem also that animals differ greatly in their susceptibility. Even if subemetic doses can be tolerated for a long time without apparent injury to health, serious consideration must be given to the experimental findings that the rate of growth may be affected, and an ample margin of safety should be insisted upon. The zinc naturally present in food is, very roughly, about four times as much as copper, and the amounts found to be toxic to animals are also roughly four or five times those of copper.

Perhaps, therefore, the limits appropriate to copper in food, increased fourfold, may be taken as a guide for zinc.

Galvanised iron vessels, owing to the ease with which zinc is dissolved from them and the danger of food becoming thereby actively emetic, should not be used where there is any likelihood of their being acted on by food. Zinc foil, preferably lacquered, may perhaps be admitted in contact with non-acid foods where serious corrosion is not likely to take place, but if an inert food like chocolate can take up zinc from zinc foil it is clear that the occasions on which it can properly be used must be limited. It has been claimed that zinc can be used with safety for plant and vessels in contact with oils and fats (Sylvester 1935). A distinction must be made between block zinc and galvanised iron. The former may perhaps be used without much danger of corrosion where the food is highly colloidal (cf. Friend and Vallance p. 405), the acidity low and the time of contact short, but with galvanised iron any food containing moisture is liable to dissolve zinc by electrolytic action. The rate of solution of zinc in contact with a cathodic metal depends both on the difference in potential between the metal in question and zinc, and on the hydrogen overvoltage (p. 174) shown by the metal.

In common with other heavy metals zinc is comparatively toxic to lower animals. 2 to 5 p.p.m. in water in the form of sulphate causes serious injury to fish (Ebeling 1928), but this applies also to alum, although aluminium is in other respects a non-toxic metal. Lobsters die in sea-water which has been exposed to zinc surfaces (Atkins 1936). Tadpoles affected by exposure to water containing 2 p.p.m. of zinc will recover completely if moved to fresh water, in contrast to a similar concentration of lead which causes irreparable injury (Schwaibold and Fischler 1933).

THE DETERMINATION OF ZINC IN FOOD

DESTRUCTION OF ORGANIC MATTER. It has always been held that zinc compounds are reduced to metal on ignition with carbon and that zinc is lost by volatilisation, but Sylvester and Hughes (1936) find that dry ashing at 500° to 550° is satisfactory for small quantities of zinc in foods and that no appreciable volatilisation occurs below 900°. When a number of other constituents besides zinc have to be determined, wet oxidation is preferable.

SEPARATION OF ZINC AS SULPHIDE. Zinc may be precipitated with hydrogen sulphide from ammoniacal solutions after removal of iron and phosphates, but precipitation is slow and the zinc sulphide is often colloidal and difficult to filter. Yellow ammonium sulphide does not precipitate dilute solutions of zinc. Bodansky (1921) made use of calcium citrate to retain zinc sulphide and prevent it from passing through the filter, and Lux (1935) carried out the precipitation in presence of asbestos fibre and sulphurous acid, the asbestos and sulphur serving to retain the fine particles of zinc sulphide.

Precipitation is more often carried out in solutions acidified with acetic, formic, citric or thiocyanic acids. According to Fairhall and Richardson (1930) zinc sulphide is obtained free from iron at pH 2·5 to 4·0. The presence of one of these acids, preferably formic, not only effects separation from iron and phosphates but also makes the zinc sulphide more granular and prevents it from passing through the filter. For separation from nickel, zinc must be precipitated at an acidity not less than pH 2·4 (Kling and Lassieur 1925). Fales and Ware (1919) have given the conditions most suitable for precipitation of zinc sulphide in presence of formic acid. Zinc can also be separated as sulphide from solutions containing not more than 0·18 per cent of free sulphuric acid (Hackl 1924).

In a method suggested by the Association of Official Agricultural Chemists for copper and zinc in gelatin the filtrate, after separation of cupric sulphide, is boiled to remove hydrogen sulphide, treated with ammonia and then made decidedly acid with formic acid. Any insoluble matter such as alumina is removed by filtering the hot solution, and zinc is precipitated with hydrogen sulphide. The solution and precipitate are heated for 15 minutes on a steam bath and allowed to stand for another 30 minutes. The liquid is filtered and the sulphide washed with a 2 per cent solution of ammonium thiocyanate, ignited and weighed as zinc oxide.

As an alternative to weighing, zinc sulphide may be brought into solution and determined by one or other of the titration or nephelometric methods with ferro- or ferricyanide as follows.

METHODS USING FERRO- OR FERRICYANIDE

Titration with Ferrocyanide. When carried out in presence of mineral acids this titration follows the equation,

$$3ZnCl_2 + 2K_4Fe(CN)_6 = 6KCl + K_2Zn_3[Fe(CN)_6]_2$$

In acetic acid solution, according to Sylvester and Hughes (1936) the product of the reaction is zinc ferrocyanide, $Zn_2Fe(CN)_6$. Normally the end-point is indicated by a brown colour with uranyl acetate on a spot plate. Sylvester and Hughes use as an internal indicator the dark blue colour given by zinc with diphenyl-benzidine in presence of ferricyanide. They find that in acetic acid in presence of *iso*propyl alcohol the end-point, shown by a change from blue to yellow, is fairly sharp, but the method is suitable only for amounts of zinc above 0·2 mg. It is affected by the presence of cadmium and manganese (Olivier 1921) and relatively large amounts of bismuth; iron also must be absent or it will obscure the change of colour at the end-point. The zinc residue obtained from the ash of a food is taken up with diluted acetic acid and *iso*propyl alcohol, a few drops of solutions of diphenyl-benzidine and ferricyanide added and the liquid titrated with ferrocyanide until the blue colour is discharged.

Von der Heide (1925) uses potassium ferrocyanide with tannin and gelatin to precipitate zinc together with other metals in cider. The liquid is filtered, the filter-paper and precipitate ashed in a silica dish and the metals present determined in the residue. Durham (1937) uses a similar method. Sweet cider must be completely fermented before addition of potassium ferrocyanide.

Nephelometric Determination with Ferrocyanide. The zinc solution containing a small amount of free mineral acid is transferred to a 50-ml. Nessler tube containing 5 ml. of 2 per cent potassium ferrocyanide. The solution becomes turbid from precipitation of the double ferrocyanide of zinc and potassium. To another Nessler tube, containing 5 ml. of the ferrocyanide solution diluted to about 40 ml., is added gradually with stirring a standard solution of zinc sulphate in dilute sulphuric acid containing 0·1 per cent of zinc until the turbidities in the two glasses match.

According to Fairhall and Richardson (1930) this method is accurate to ± 0·06 mg. but only within narrow limits of acidity and salt concentration. The most favourable degree of acidity corresponds to pH 2·3 in a solution containing 0·2 per cent of potassium sulphate. Full development of opalescence is reached in about 10 minutes. The best degree of turbidity for matching is given with 0·2 to 0·25 mg. of zinc in 50 ml. of solution.

Iodimetric with Ferricyanide. This method was introduced by Lang (1929). Potassium ferricyanide oxidises potassium iodide in solutions containing much mineral acid and sets free iodine. This is a well-known method of determining ferricyanides. In neutral solutions, however, or in presence of a small quantity of acetic acid, ferricyanide does not oxidise potassium iodide unless a zinc salt is added. Presumably the active agent is the double zinc potassium ferricyanide which is first formed and which becomes reduced to zinc potassium ferrocyanide with the liberation of an equivalent amount of iodine,

$$2K_3Fe(CN)_6 + 2KI + 3ZnSO_4 = K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4 + I_2$$

For very small quantities of zinc, such as may be present in the residue from the dithizone extraction of the ash of a food, Sylvester and Hughes (1936) proceed as follows :

Glacial acetic acid (0·1 ml.) is added to the residue and about 0·01 g. of ammonium hydrogen fluoride followed by 2 ml. of 5 per cent potassium iodide solution and two drops of 1 per cent starch solution. If a blue colour appears after the addition of starch; 0·002N sodium thiosulphate solution is added until the colour is just discharged. About 0·5 ml. of potassium ferricyanide solution (1 per cent) is then added and the liberated iodine titrated with 0·002N sodium thiosulphate solution. The object of adding fluoride is to immobilise any traces of iron which may be present and so to prevent the formation of Prussian blue. The blue starch-iodine colour may be adsorbed by the precipitated zinc ferrocyanide, and thus the precipitate serves as an indicator. 0·51 ml. of 0·002N sodium thiosulphate solution is equivalent to 0·10 mg. of zinc or 1 ml. = 0·196 mg. of zinc. Sahyun and Feldkamp (1936) use this reaction for determining zinc in trichloracetic acid extracts of biological material.

METHODS INVOLVING THE USE OF DIPHENYLTHIOCARBAZONE (DITHIZONE). Diphenyl-thiocarbazone or 'dithizone', $C_6H_5N : N.CS.NH.NH.C_6H_5$, has been used (*a*) as a means of separating zinc, together with other heavy metals, from phosphates of calcium and iron and other constituents of food ash and also (*b*) for the actual colorimetric determination of zinc after removal of interfering metals.

(*a*) *Separation of Zinc with Dithizone.* In Sylvester and Hughes' method (1936) the solution of the ash is brought to a degree of acidity corresponding to pH 4·5 by addition of ammonium acetate and extracted several times with dithizone in chloroform. This treatment takes out zinc, copper, mercury, silver, bismuth and cadmium in the form of complexes with dithizone. The chloroform extract is then washed with dilute hydrochloric acid which removes zinc, bismuth and cadmium and precipitates silver. The acid extract is evaporated to dryness and organic matter destroyed by oxidation with perchloric acid and hydrogen peroxide. Zinc is determined in the residue by titration with ferrocyanide or iodimetrically after adding ferricyanide. Perlman and Mensching (1937) determine zinc in maple syrup by heating it with dilute nitric acid without destroying organic matter. Citric acid is then added to de-ionise iron, the solution made slightly alkaline with ammonia and a dithizone extraction carried out on this weak ammoniacal solution instead of at pH 4·5 as in Sylvester and Hughes' method. Zinc is extracted from the dithizone-chloroform with dilute nitric acid, any copper present remaining in the chloroform layer in combination with dithizone. The nitric acid solution is evaporated to dryness and zinc in the residue determined iodimetrically in presence of ferricyanide. The other metals extracted by dithizone do not interfere in the iodimetric titration.

(*b*) *Colorimetric Determination as Zinc-dithizone Complex.* The zinc complex with dithizone is cherry-red in chloroform solution. Somewhat similar colours are given by several other metals which must be separated before zinc can be determined. The colorimetric determination of zinc may therefore be conveniently dealt with as part of a scheme for the determination of copper, lead and zinc in the same sample.

DETERMINATION OF ZINC, COPPER AND LEAD IN FOODS WITH DITHIZONE. Strohecker and Riffart (1938) proceed by first extracting copper with dithizone from a solution of the ash in 0·2 per cent sulphuric acid. Copper is the only metal which can thus be extracted from acid solution (p. 48) except mercury and silver, which are rarely present in foods in significant amounts. Ammonium citrate is then added to the solution to de-ionise iron and prevent precipitation of phosphates. The solution is made alkaline with sodium hydroxide and potassium cyanide is added. A second extraction with dithizone now takes out lead, zinc being retained in aqueous solution by cyanide. The cyanide solution is then acidified, the hydrogen cyanide driven off by heating and the solution brought to an acidity of about pH 4·0 with ammonium acetate. A third extraction with dithizone now takes out zinc.

The three dithizone extracts containing respectively copper, lead and zinc are examined in a photometer using coloured screens and compared with standards.

Holland and Ritchie (1939) proceed on rather different lines. They find that sodium diethyl-dithiocarbamate (a reagent used for the determination of copper, p. 49) forms precipitates in dilute ammoniacal solution with all the common metals except zinc, and prevents these from reacting with dithizone although the compounds are soluble in chloroform. If, therefore, we add sodium diethyl-dithiocarbamate to an ammoniacal solution containing zinc with or without other metals and then extract with dithizone in chloroform, we get a red colour which is specific for zinc. The copper compound with diethyl-dithiocarbamate gives a yellow solution in chloroform so that copper, if present in considerable amount, must be removed. Holland and Ritchie first extract copper, lead and zinc together from slightly ammoniacal solution with dithizone in chloroform. From the chloroform-dithizone extract lead and zinc are removed by washing with dilute hydrochloric acid, leaving copper which is separately determined. The acid extract containing lead and zinc is divided into two parts. In one part lead is immobilised by adding ammonia and diethyl-dithiocarbamate, and zinc is extracted with dithizone and determined colorimetrically by comparison with standards in a Dubosc colorimeter using a green colour filter. In the other part zinc is immobilised with cyanide after adding ammonia, and lead is extracted with dithizone and determined colorimetrically in a similar manner.

Cowling and Miller (1941) find that diethyl-dithiocarbamate reacts to some extent even with zinc, but that if the alkalinity and volume of the solution and the amounts of carbamate and of dithizone are the same in the test and in the control the determination of zinc is accurate. Cholak and others (1943) get a sharper separation by using di-β-naphthyl-thiocarbazone instead of dithizone. It should be noted that in neutral, as opposed to alkaline, solution zinc gives a precipitate with sodium diethyl-dithiocarbamate. This in fact forms the basis of a turbidimetric method of determining zinc in water (Atkins 1935).

Hibbard (1937, 1938) describes a method of titrating zinc dithizone with bromine solution.

The following method, similar in principle to that of Holland and Ritchie, has been found by me to be satisfactory for the determination of copper, zinc and lead in small samples of most kinds of food. It is a variant of the so-called 'titrimetric extraction' method used by various workers and obviates the use of a colorimeter and the preparation of standards. In its application to lead it is similar to the method adopted by Fischer and Leopoldi (p. 92).

Separation of Copper from Lead and Zinc. A suitable weight of material, usually 5 g., is oxidised in a round-bottomed flask, preferably of Pyrex glass, using 5 ml. of sulphuric acid and the minimum quantity of nitric acid. Residual nitric acid is expelled as far as possible by diluting with about 20 ml. of water and reheating until the sulphuric acid fumes freely. The solution is again diluted with about 20 ml. of water, and any residue is separated either by centrifuging or filtration, extracted with hot diluted hydrochloric acid (1 : 1) and washed with hot water. The acid extracts and washings are added to the main filtrate.

2 g. of citric acid are dissolved in the liquid and ammonia solution (0·880) added until the acid is neutralised and the mixture contains an excess of about 0·5 ml. of the ammonia solution. The liquid is transferred to a 250-ml. separator and the volume with washings adjusted to about 100 ml. Lead, copper and zinc are then extracted with a 0·1 per cent solution of dithizone in chloroform. Three extractions are usually enough, using 10 ml. for the first and 5 ml. for the second and third. The dithizone extracts are washed in turn with about 10 ml. of water in a smaller separator, and the combined dithizone extracts are collected in a third separator. Lead and zinc are separated from copper by extracting the dithizone solution twice with dilute hydrochloric acid, using a mixture of 20 ml. of water with 2 ml. of normal hydrochloric acid solution for each extraction. Traces of residual dithizone are removed from the acid liquor by shaking with chloroform. The acid extracts and washings are boiled to remove chloroform, made up to 50 ml. and reserved for the determination of zinc and lead.

A control or blank determination is carried through with similar apparatus and quantities of reagents, omitting only the actual material under examination.

Determination of Copper. Details of this determination have already been given on page 52. The dithizone solution containing copper is transferred to a 6″ × 1″ test-tube, preferably of Pyrex glass, and the solvent evaporated. The residue is oxidised with 0·5 to 1 ml. of sulphuric acid and a little nitric acid, residual nitric acid being expelled as before. The acid liquor is cooled and diluted and the whole or a measured part taken for the determination of copper.

To the solution is added 1 ml. of 5 per cent. citric acid and enough ammonia solution (0·880) to neutralise the acids and give an excess of about 2 ml. The ammoniacal liquid is transferred to a 50-ml. colourless glass Nessler cylinder and mixed with 5 ml. of 1 per cent gum arabic solution. A second cylinder is prepared containing a similar measured part from the control experiment on the apparatus and reagents. To each are then added 5 ml. of a freshly prepared 0·2 per cent aqueous solution of sodium diethyl-dithiocarbamate and the contents mixed by inverting the tubes. The colour due to copper in the test cylinder is then matched by adding a standard solution of copper sulphate (1 ml. = 0·01 mg. copper) to the control.

Determination of Zinc. So much of the acid solution as will contain about 0·01 to 0·02 mg. of zinc is transferred to a 100-ml. pear-shaped separator, preferably of Pyrex glass, and if necessary diluted to 20 ml. Into a second separator is introduced a similar quantity of the control solution. To each is added sufficient normal ammonia solution to neutralise the free acid and leave an excess of 2 ml. of normal ammonia solution. 2·5 ml. of 0·2 per cent sodium diethyl-dithiocarbamate are then added to each. A weak solution of dithizone in chloroform (2 ml. of approximately 0·1 per cent solution diluted shortly before use to 50 ml. with chloroform) is added to the test solution with shaking until the red colour first produced gives place to a purple-violet. A similar volume of the weak dithizone solution is then added to the control. Both separators are well shaken for about ¼ to ½ minute. Standard zinc sulphate solution (1 ml. = 0·01 mg. zinc) is then added gradually to the control until, on shaking, the colours in the two separators match exactly.

Great care must be taken to avoid errors due to traces of zinc derived from reagents and glass-ware. The control should be carried through from start to finish in a manner closely similar to that of the test.

Determination of Lead. A measured portion of the acid solution, usually 20 ml. or more, is transferred to a separator as for the zinc determination. A second separator is prepared with a similar portion of the control. Normal ammonia solution is added to each until the acid is neutralised and the liquid contains an excess of 0·5 ml. of normal ammonia solution for every 20 ml. of liquid. To each are added 10 drops of 10 per cent potassium cyanide solution. Weak dithizone solution (1 ml. of approximately 0·1 per cent solution diluted to 50 ml. with chloroform) is added to the test liquid until, on shaking, the chloroform layer changes to a purple-violet. After adding a similar quantity of dithizone solution to the control, a standard solution of lead (1 ml. = 0·01 mg. lead) is run in gradually until, on shaking, the colours of the chloroform globules in each separator match exactly.

With quantities up to 0·02 mg. of each of the metals (2 ml. of the standard solution) the accuracy of the method is for copper ± 2·5 per cent, for lead ± 7·5 per cent and for zinc ± 10 per cent.

Strafford, Wyatt and Kershaw (1945) have given a scheme for the successive determination of minute amounts of copper, arsenic, lead, zinc and iron in medicinal products. They have introduced a new reagent, diethylammonium diethyl-dithiocarbamate (p. 188), which has certain advantages over the sodium salt. They find also that when toluene is used as a solvent for dithizone instead of chloroform, the excess of dithizone used in the extraction is more readily and completely removed by washing with a weakly alkaline aqueous solution, leaving only the metal dithizone compound in the toluene. This facilitates colorimetric comparison of the extracts. A further device introduced by them is the addition of formaldehyde to the cyanide zinc solution. The cyanide is thus converted into formaldehyde cyanhydrin, and the zinc can be extracted with dithizone. They determine the optical density of the coloured solutions in a Spekker photo-electric absorptiometer.

In the standard method recommended by the Analytical Methods Committee of the Society of Public Analysts (1948) the sample is subjected to wet oxidation, and the solution neutralised to pH 7·5–8·0 and shaken with diethylammonium diethyl-dithiocarbamate in chloroform. The diethyl-dithiocarbamate of zinc is thus extracted, together with those of lead, copper, silver, bismuth, cadmium, mercury, nickel and cobalt if any of these are present. From this extract zinc is separated by shaking with a solution of sodium citrate in N sodium hydroxide. The alkaline solution containing zinc is acidified and oxidised with bromine water. After neutralisation with ammonia, the pH of the solution is fixed at 8·7–9·3 by the addition of sodium borate solution. Zinc is then determined by titrimetric extraction (p. 92) with a chloroform solution of dithizone which has previously been standardised against a zinc solution.

In Taylor and Alexander's method (1945) the difficulties of effecting a sharp separation of zinc from other metals with dithizone are overcome by treating the solution first with hydrogen sulphide which separates all the metals of that group, then removing nickel and cobalt with dimethylglyoxime and α-nitroso-β-naphthol, after which zinc is determined by dithizone extraction from a slightly ammoniacal citrate solution at about pH 8·5.

REACTIONS OF THE COMMONER METALS WITH DITHIZONE. In the following table the sign + signifies that the metal in question is extracted as its dithizone complex by a solution of dithizone in chloroform.

REACTIONS OF METALS WITH DITHIZONE

	Ammoniacal solution	*pH 4·0 to 4·5*	*Strongly acid solution*	*Strongly acid solution + KI*	*Ammoniacal cyanide solution*	*Ammoniacal solution containing sodium diethyl-dithio-carbamate*
Copper . .	+	+	+	+		
Lead . .	+				+	
Zinc . .	+	+				+
Bismuth .	+	+	*		*	
Cadmium .	+	+				
Cobalt . .	+					
Iron (ferrous)	+					
Mercury .	+	+	+			
Nickel . .	+					
Silver . .	+	+	+			
Tin (stannous)	+	+			+	
Manganese .	+					
Thallium .	+				+	

* Bismuth is retained in aqueous solution by a large excess of cyanide. In presence of small amounts of cyanide it may be partially extracted by dithizone. Bismuth can also be extracted from an acid solution at pH 2·0 by shaking with a large excess of dithizone in chloroform.

See page 283 for remarks on nickel and cobalt.

ZINC AMMONIUM PHOSPHATE METHOD. Zinc can be separated as $ZnNH_4PO_4$, and the phosphoric acid in the precipitate determined colorimetrically. The method, as originally introduced for the determination of inorganic phosphorus in biological material by Bell and Doisy (1920), depends on the fact that a mixture of hydroquinone and sulphur dioxide (or other reducing agents) will reduce phosphomolybdic acid to give a blue reduction product, $(Mo_2O_5, 4MoO_3)_2H_3PO_4$, which is stable on dilution. When a mixture of molybdic acid and phosphomolybdic acid is reduced the intensity of the blue colour is therefore proportional to the amount of phosphoric acid present (p. 198).

Fiske and Subbarow (1925) improved Bell and Doisy's method by substituting 1:2:4-aminonaphthol sulphonic acid for hydroquinone as the reducing agent, and Todd and Elvehjem (1932) applied it to the determination of zinc. Zinc is first separated by co-precipitation with added copper as sulphide. Copper is removed with hydrogen sulphide in acid solution and zinc precipitated as zinc ammonium phosphate, $ZnNH_4PO_4$, under carefully controlled conditions. Phosphoric acid in the precipitate is then determined by conversion into ammonium phosphomolybdate and reduction of this with 1:2:4-aminonaphthol sulphonic acid and sodium bisulphite. The blue colour obtained is compared with standards. The zinc content of the sample is calculated from the amount of phosphoric acid found.

DETERMINATION OF ZINC WITH 8-HYDROXYQUINOLINE. Zinc is precipitated by 8-hydroxyquinoline ('oxine') from acid acetate, ammoniacal or alkaline tartrate solution as the greenish-yellow crystalline compound $Zn(C_9H_6ON)_2, 2H_2O$. According to Berg (1927), precipitation in acetic acid-acetate solution allows of a separation from magnesium and other alkaline earths but not from copper or cadmium. By precipitation in alkaline tartrate solution zinc can be separated from ferric iron, cobalt, nickel, aluminium, chromium and the metals of the hydrogen sulphide group except copper, but from mercury only if the solution contains cyanide.

The zinc oxine precipitate can either be weighed or titrated with standard bromide-bromate solution using indigo-carmine as indicator. A small additional quantity of bromide-bromate solution is added after the end-point has been reached and this excess determined by adding potassium iodide and titrating the liberated iodine with thiosulphate. Hamence (1937) determines zinc by this method after removal of copper as sulphide. Zinc is precipitated in ammoniacal ammonium acetate solution at about pH 7·5 and the precipitate titrated with N/50 bromide-bromate solution. 1 ml. of N/50 bromide-bromate solution = 0·163 mg. of zinc, the hydroxyquinoline being brominated to give 5:7-dibromo-hydroxyquinoline, according to the equation :

$$C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr$$

For full details of the 8-hydroxyquinoline method reference should be made to Berg's and Hamence's papers.

DETERMINATION AS ZINC QUINALDINATE. In

this method, due to Allport and Moon (1939), the material is ashed at 500° to 550° and zinc extracted with dithizone in chloroform from an ammoniacal solution containing resorcinol, which is found to facilitate the extraction of minute traces of the metal. Zinc is then removed from the dithizone solution with dilute hydrochloric acid, heavy metals are precipitated with hydrogen sulphide, hydrochloric acid is replaced by acetic acid and a solution of sodium quinaldinate (α-quinoline carboxylate) gradually added. The precipitated zinc quinaldinate may be weighed on a micro-balance or determined colorimetrically by means of the yellow colour given with phthalic anhydride on reduction.

OTHER METHODS OF DETERMINING ZINC IN FOOD. Several other methods for the determination of zinc in foods have been described. Electrolysis can be carried out in alkaline solution, preferably in presence of cyanide, with a current density of 0·5 to 1 ampere per square decimetre, also in neutral or acetic acid solution. If a platinum cathode is used it should first be coated with a protective film of copper. Breisch (1924) uses a silver-gauze cathode with a rotary stirrer and a current of 3 amperes. Nitrates interfere and deposition of zinc does not begin until they have been completely reduced to ammonia. Neumann as long ago as 1908 claimed to be able to detect 0·0016 mg. of zinc by micro-electrolysis in 0·1N potassium hydroxide using a small platinum bead as anode and a highly polished thin copper wire as cathode.

Zinc can be determined by precipitation as mercury zinc thiocyanate, $HgZn(CNS)_4$ (Kirby 1925), a violet crystalline compound which affords a specific but not very sensitive test ; as 5-nitroquinaldinate (Lott 1938) ; and as the very insoluble crystalline calcium zincate (Bertrand and Javillier 1907) ; also colorimetrically by means of the green fluorescence given with urobilin (Lutz 1925). Lux (1935) determines zinc as metal by distillation of the oxide in hydrogen and claims to be able in this way to determine small fractions of a milligram in biological material (cf. distillation of mercury, p. 461).

Zinc can also be determined by spectrographic and polarographic methods (p. 96). The latter as applied to zinc in plant tissues has been described by Walkley (1942).

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4

[TIN]

TIN is found in traces in most soils and very small amounts occur naturally in plant and animal tissues. As far as is known it has no biological function. The presence of larger quantities in food and in the human body is due for the most part to its use as a protective coating for metals which may come into contact with foods, especially tin-plate for canning.

TIN-PLATE AND ITS EXAMINATION. The steel plate generally used for making cans for food preserving is of 30 gauge or 0·31 mm. in thickness. The weight of the tin coating is expressed in the trade as the weight of tin per ‘ base box ’. The base box is essentially a unit of area and contains the equivalent of 112 sheets of steel plate measuring 20 × 14 inches, representing a total area of 31,360 square inches or 62,720 counting both sides. A base box of 30-gauge steel plate weighs 107 lb. and normally 1½ to 1¾ lb. of tin is used to coat it, corresponding to about 17 to 20 g. of tin per square metre on each side of the plate. The thickness of the coating on each side is of the order of 0·0025 mm. About one-seventh to one-ninth of this tin is in the form of an intermediate layer of a tin-iron alloy, $FeSn_2$. The amount of tin in the alloy layer is fairly constant at 3 oz. per base box, so that with the heavier tin coatings the proportion of alloyed tin is smaller (Clarke 1934).

The tin coating varies somewhat in thickness in different parts of the plate and is never quite free from small holes which permit corrosion by exposure of the underlying steel. These pinholes can be detected by the ferrocyanide test. In Serger's modification of this test (1926) the sample of tin-plate is treated for two minutes in a shallow dish with 5 per cent sulphuric acid solution and then rinsed with water. It is placed in another shallow dish with a warm solution containing 1 per cent of potassium ferrocyanide, 2·5 per cent of glycerol and 7·5 per cent of gelatin, and the solution allowed to set and remain undisturbed for 24 hours. A glass plate ruled with centimetre squares is then placed on the surface of the gelatin and the number of blue spots in each square counted. The sheet is of good quality when it shows only 1 to 3 spots per $cm.^2$, of medium quality with 4 to 12 spots, and of poor quality if it shows more than 12. Macnaughtan, Clarke and Prytherch have devised a standard hot-water test in which pinholes are shown up by the spots of rust which form on them. Kerr (1942) gives a test applicable to thin electrolytic tin coatings, in which the plate is immersed for a standard time in a solution containing acetic acid and thiocyanate, and the amount of ferric thiocyanate formed is determined colorimetrically.

The porosity of tin-plate can be reduced or eliminated by electrodeposition of tin from a sodium stannate or acid stannic sulphate solution. The coating is much thinner than that of dipped tin-plate. It is suitable for articles which do not require soldering, but it is not well adapted to food canning since if the deposit is thinner than 0·0001 inch, corresponding to about 1½ lb. per base box, difficulty may be experienced in soldering (cf. Colgate 1945).

The determination of the amount of tin in tin-plate may be carried out by heating a representative sample of the plate with concentrated hydrochloric acid in a current of carbon dioxide and titrating immediately with standard iodine solution and starch as indicator. The ferrous iron present does not interfere with the titration. Other methods depend upon the removal of tin from a weighed sample of tin-plate and weighing the residual iron after washing and drying it. The tin coating can be removed in several ways.

(*a*) By repeated treatment with sodium peroxide and a little water at 80° or with sodium peroxide and perhydrol (Meyer 1909).

(*b*) By immersion of the tin-plate, after removal of grease, in 29 per cent hydrochloric acid (sp. gr. 1·15) containing 2 per cent of antimony trioxide, which dissolves the tin coating rapidly without affecting the iron. Tin may be dissolved from a selected area if the rest of the plate is protected with cellulose varnish. The antimony reagent dissolves also the underlying coating of tin-iron alloy, $FeSn_2$, and a correction is necessary for the iron thus dissolved. For hot-dipped plate of about 1·3 lb. per base box this correction is about 5 per cent of the tin and for electro-deposited tin-plate only one-quarter of this (Clarke 1934).

(*c*) By heating the tin-plate with a solution of sodium plumbite (Kohman and Sanborn 1927). This method removes only the unalloyed tin and leaves the layer of $FeSn_2$ untouched.

(*d*) By immersing the plate in anhydrous stannic chloride saturated with chlorine (Masel 1929). This removes tin but not the alloy layer, and a correction of 0·1 per cent of the weight of the tin-plate is made for undissolved tin in this layer.

(*e*) By electrolysis with the tin-plate as anode in a solution of sodium nitrate. This is a quick and convenient method (Heise and Clementi 1916). A piece of tin-plate of known weight and area (about 1 square inch) is made the anode in a small beaker containing sodium nitrate solution of roughly 2½ per cent strength. The cathode is also of tin-plate bent round the central anode so as to distribute the current on both sides. A current of about 0·5 ampere is passed through the cell. The tin becomes oxidised and detached from the anode as insoluble metastannic acid and collects at the bottom of the beaker leaving the iron quite clean. A small amount of iron is found in the liquid owing to removal of the $FeSn_2$ alloy layer, but the steel plate itself is not attacked. The whole operation is completed in a few minutes.

Chalmers, Hoare and Tait (1937) describe magnetic methods by which the thickness of the tin coating at any point can be measured without damaging the tin-plate.

Block tin often contains from 0·02 to 0·04 per cent of arsenic, which is too small a quantity to give rise to appreciable contamination of food. In all probability the tin coating contains less arsenic than the steel plate.

The use of tin-lead alloys for plating cooking vessels has been referred to on page 74. Alloys of tin with zinc and with copper are now deposited electrolytically upon steel plate, and may possibly be met with in food containers or cooking vessels (Hedges 1945).

CORROSION OF TIN-PLATE. The corrosion of tin-plate in food containers depends upon several factors. In the first place the small quantity of air unavoidably present in the head-space of the can promotes the solution of tin. Chapman (1913) found that tartaric acid solution had very little action upon pure tin in absence of oxygen, but attacked it readily if oxygen were present. Other organic acids have a similar but somewhat less intense action. After a short time all the oxygen in the head-space of the can has been used up, and further solution of tin and of any exposed iron is largely governed by the action of the tin-iron couple. Normally tin is cathodic to iron, for instance when a tin-iron couple is immersed in dilute sulphuric acid. Positive stannous ions are active close to the surface of the tin, and these tend to prevent the solution of more tin and to maintain its potential at a point higher than that of iron. In contact with foods these stannous ions form complexes with organic acids and cease to function as cations, thus allowing more stannous ions to pass into solution, and the tin becomes anodic to iron (Hoar 1934). It dissolves in preference to iron, and iron being now the cathode does not go into solution. Moreover, in a plain unlacquered can, tin occupies a large area from which it slowly dissolves while iron is exposed only at pinholes, so that the protective action of a large tin anode is exerted at a few cathodic points (Morris and Bryan 1931, 1936).

Any condition which favours the removal of stannous ions from solution makes it possible for more tin to dissolve. The canner tries to reduce corrosion and solution of tin as far as possible while still keeping it anodic to iron. In general, the less acid the food the more readily are organic complexes formed with stannous tin, thus removing it from solution, and corrosion and solution of tin are therefore greater, as a rule, in the less acid foods. It is a common practice to add citric acid to weakly acid fruits in order to reduce corrosion. Normally, corrosion of tin is greatest in fruit products at about pH 4·0. Oxidising agents accelerate it, since they oxidise and remove stannous ions and thus enable more tin to pass into solution. Ferric salts oxidise stannous salts the more readily the more acid the solution, so that if ferric salts are present tin is more easily corroded by highly acid foods. Other oxidising agents which accelerate corrosion of tin are nitrates and cupric salts; vegetables greened with copper salts attack tin-plate strongly (p. 26). Oxidising agents may also operate by removing hydrogen from points where the iron is exposed, thus lessening the effect of hydrogen polarisation. Anthocyanin pigments, monomethylamine in canned shrimps, sulphur dioxide and other sulphur compounds also act as accelerators of corrosion, while tin salts in solution, sugars and colloids such as gelatin retard it. The action of these various agents in promoting or depressing the corrosion of tin would seem to depend upon whether they accelerate or retard the removal, by oxidation or otherwise, of stannous ions from solution, or of hydrogen from any exposed iron plate (Morris and Bryan 1931, 1936).

When foods cannot be packed in plain cans owing to corrosion, lacquered tin-plate is used. Hedges (1937) gives details of the types of lacquers used and the way in which they are applied to the metal. Lacquers usually consist of either copal or a synthetic resin with a tung-oil or linseed-oil vehicle, a mineral-oil filler and a manganese or cobalt drier. For some foods vegetable-oil vehicles are replaced by alcohols, ketones or hydrocarbons. The lacquering of tin-plate is usually done on the flat since it requires stoving, and there are difficulties in the way of stoving the finished can bodies. Hirst and Adam (1939), however, advocate the use of post-lacquered cans which are now available to canners (cf. Farrow and Green 1941). In making up and seaming cans made from pre-lacquered plate the lacquer coating is often scratched and weakened in places, especially along the seams. Thus although the lacquer protects by far the greater area of the can, there may be a few points at which the tin-plate is exposed and at which the action of corrosive agents in the food can be concentrated. The first result is that under the influence of residual air in the can, the tin at these points is dissolved and the underlying iron exposed. Since the rest of the surface of the tin is covered with lacquer, no electrochemical protection is afforded to the iron at the points where it is exposed, and it dissolves in the organic acids of the food with production of hydrogen which bulges the ends of the can and forms what is known as a ‘ hydrogen swell ’. If the action is prolonged the can becomes perforated. Hydrogen swells are the cause of most of the spoilage of canned fruits both in the United States (Kohman 1925, 1929) and in this country.

Hydrogen swells are by no means confined to lacquered cans. They may occur in plain cans but are far more frequent in lacquered. Hoar, Morris and Adam (1939) find that the composition of the steel base is of significance in the formation of hydrogen swells, steel with high copper and low phosphorus content being comparatively resistant to corrosion by fruit acids.

The addition of 0·5 per cent of antimony or 0·4 per cent of bismuth to tin increases its resistance to attack by citric acid (Hanson and Sandford 1935, Morris and Bryan 1936). This may be of significance to the analyst when examining foods for lead, since some of the methods used for lead do not differentiate between lead and bismuth, and it is assumed that the latter metal is rarely present in food.

DISCOLORATION OF CANNED FOOD. The colour of many fruits is affected by tin-plate owing to combination of tin with anthocyanin colouring matters, giving blue or violet ‘ lakes ’. Black currants are particularly prone to this colour change and, in general, red or purple-coloured fruits can be packed only in lacquered cans. Tomatoes are an exception since their colouring matter is a lipochrome, not an anthocyanin. Hedges (1937) refers also to opalescence or muddiness of fruit syrups caused by oxides or basic salts of tin and to the bleaching action of nascent hydrogen produced in the can. This bleaching action is particularly liable to affect artificial colours added to food. Iron also may cause blackening by combination with tannins if oxygen is present, or else by formation of iron sulphide (p. 253).

Tin readily causes haze or turbidity in beer and should be avoided in brewing equipment, especially in places where it is likely to be corroded by detergents in cleaning operations (Raux 1937, Matthews 1929). Sulphur haze can also occur in liquids containing sulphur dioxide in contact with tin (p. 115).

The surface of tin-plate often becomes brown owing to the action of sulphur compounds in the food. Kerr (1940) finds that treatment of the plate with an alkaline solution containing sodium phosphate and sodium dichromate prevents this discoloration with vegetable and meat products but not with fruits.

TIN IN FOOD. Not much information is available on the natural occurrence of tin in vegetable foods, but it can be shown by spectrographic methods to occur in traces in most foods. Kent (1942) found 1·2 p.p.m. in wheat flour and 1·6 p.p.m. in bran. The outer pericarp of wheat (‘ beeswing ’) contained 3·9 p.p.m. (cf. p. 97).

In Canned Food. Buchanan and Schryver (1908) examined many canned foods of all kinds and found that most of them contained less than 2 grains of tin per pound (286 p.p.m.). They suggested the adoption of this figure as a maximum limit for administrative purposes. Since that time the methods of canning have been greatly improved, especially by the use of lacquered cans for foods liable to take up tin. Adam and Horner (1937) give figures for tin in a number of canned fruits and vegetables, which show that in double-lacquered cans the tin content of the food rarely exceeds 40 p.p.m. and that even in plain cans with most foods under normal storage conditions it is usually below 100 p.p.m. Some foods are unsuitable for packing in plain cans owing to corrosion. In asparagus, for instance, Adam and Horner found tin varying from 290 to 330 p.p.m. according to length of storage. Presumably some constituent of asparagus readily removes stannous ions from solution and thus promotes corrosion of tin. Other foods liable to contain excessive amounts of tin are fish in oil, shrimps, tomato products, spinach, rhubarb, gooseberries, plums, black cherries, coppered vegetables, and foods which have been treated with sulphur dioxide or those which contain salt. Some of these after prolonged storage in plain cans may contain several hundred parts of tin per million and have on rare occasions reached 7 grains per pound or nearly 1,000 p.p.m. Lickorish (1936) found 4·75 grains per pound (680 p.p.m.) in spinach and 4·9 gr. per lb. (700 p.p.m.) in apricots. Bagnall (1935) refers to two samples of sild in olive oil containing 5·3 and 5·5 gr. per lb. (760 and 785 p.p.m.) and Williams (1935) found that 5 samples out of 70 examined contained 4 to 6·7 gr. per lb. (570 to 960 p.p.m.). Dixon found as much as 12 to 18 gr. per lb. (1,720 to 2,570 p.p.m.) in anchovies with tomato sauce in unlacquered cans, and large amounts also in tomato purée.

Adam and Horner (1937) point out that in cans made from tin-plate containing 1¾ lb. of tin per base box the tin on the inner surface of a 30-oz. container would be enough, if completely dissolved and absorbed by the contents, to give a concentration of about 1,300 p.p.m. With smaller containers the proportion would be higher owing to the surface area being relatively greater compared with the bulk of the food.

Drummond and Macara (1938) examined some canned foods which had been kept from the time of Sir William Parry's Arctic expedition in 1824 and found very large amounts of tin, over 2,400 p.p.m. in one sample. The tin coating was very heavy, 8 to 10 lb. per base box.

The tin content of food may increase considerably if the can is opened and the food allowed to remain in it exposed to the air. In one such instance the tin in a sample of canned fish rose in 12 days from 154 to 420 p.p.m. (Glassmann and Barzutzkaja 1928).

Scott and Stewart (1944) have made the observation that *Clostridium botulinum*, the bacillus of botulism, will not grow in canned foods containing considerable amounts of dissolved tin. For canned beetroot the critical concentration of tin, added in the form of stannous citrate, was 150 p.p.m. and for carrots 30 to 60 p.p.m. This indicates that plain cans may be preferable to lacquered for vegetables, which, owing to their low acidity, are more likely than canned fruits to favour the growth of this bacillus.

Tin in Processed Cheese. It is customary to wrap processed crustless cheese in tin-foil to keep it moist and in good condition, and the tin is liable to become corroded and dissolved by lactic and citric acids in the processed cheese. Dyer and Taylor (1931) found from 315 to 786 p.p.m. in a few samples of Gruyère cheese, but the greater number of samples examined contained less than 2 gr. per lb (286 p.p.m.). As much as 2,000 p.p.m. has been recorded in a sample of cheese condemned as being badly blackened and mouldy. In one instance Elten (1929) found 2 per cent of tin in the layer of cheese immediately in contact with the foil. Eckardt (1909) found that Neuchâtel and Camembert cheeses in tin-foil contained not more than 150 to 300 p.p.m., but that on re-wrapping them and keeping them for a further ten days the tin content rose to as much as 850 to 2,000 p.p.m. This is similar to the large increase in the amount of tin dissolved by food from cans which are left open to the air.

The tin-foil used for wrapping cheese is often alloyed with 2 to 4 per cent of antimony, which is left after solution of the tin as a black or brown stain on the cheese and may result in bad discoloration of the outer layers.

Tin in Demerara Sugar. Tin chloride has been used in the manufacture of Demerara sugar for over 60 years, to stabilise the clear brownish-yellow colour of the crystals (*Ann. Repts. C.M.O.* 1929, 1936). The colouring matter of the crude sugar is saccharetin, the constitution of which has not been fully investigated. Tin salts appear to form a stable 'lake' with this colouring matter and prevent it from becoming bleached on storage. Tin is added to the sugar before crystallisation in the form of a 50 per cent solution of stannous chloride. This solution has a specific gravity of 1·6 and is added at the rate of 1 gallon to 5 tons of massecuite or 6 lb. per ton of finished sugar. The bulk of this tin remains in the residual liquor from the crystallisation and accumulates in the molasses. The finished sugar, with a small quantity of adhering molasses, should not contain more than 0·3 gr. per lb. (43 p.p.m.), but many samples contain over 1 gr. per lb. (143 p.p.m.), and some have shown 3 gr. or even more (*Ann. Rept. C.M.O.* 1936). If Demerara sugar is moistened with the juice of black currants or blackberries a pronounced change of colour to blue or violet takes place after a few minutes owing to the action of tin in the sugar on the colouring matter of the fruit. Iron gives a closely similar colour but is not likely to be present in amount sufficient to interfere with the test. It may be noted that the Royal Commission on Arsenical Poisoning in 1903 considered tin chloride in relation to possible arsenical contamination of sugar and expressed the opinion that it was an objectionable and unnecessary addition to sugar and that it should no longer be used.

Tin in Confectionery. Tin forms a yellow lake with xanthorhamnin, the colouring matter of Persian berries (*Rhamnus sp.*), and this lake has been used in confectionery, as has also the tin lake of cochineal. The aluminium lakes of these colours are preferred by manufacturers, and it is doubtful whether tin lakes are now used to any appreciable extent (*Ann. Rept. C.M.O.*, 1929).

ABSORPTION AND EXCRETION OF TIN. Schryver, in experiments conducted upon himself, found that when 1 gr. (65 mg.) of tin was ingested daily for 5 days no tin was excreted in the urine, but that with 2 gr. daily for 5 days an average amount of 11 mg. was excreted. With 3 gr. (195 mg.) the daily excretion in the urine rose to about 15 mg. The remainder of the tin was excreted in the faeces, but there was evidence of some retention with 2 gr. daily (Buchanan and Schryver 1908). These results, considered in relation to those obtained by feeding experiments on animals and to the amounts of tin actually found in canned foods, led Buchanan to conclude that the presence of tin in quantities approaching 2 gr. per lb. (286 p.p.m.) may be taken to signify that the food has become potentially dangerous to health. In default of statutory standards this view has been accepted by most public health authorities, although it has been criticised by Savage (1939) on the ground that it was not warranted by the evidence available.

Against Schryver's results must be set those obtained by Schwartze and Clarke (1927) who found that men receiving 2 to 2·75 g. of tin in 5 days, equivalent to 6–8 gr. daily, showed either no tin whatever in their urine, or else less than 0·4 mg. On the other hand, Kent and McCance (1941), from metabolism experiments over a period of one week with two healthy men, have lately obtained evidence which suggests that with very small intakes of tin in food, most of it is absorbed and excreted in the urine. They found that with an average daily intake of 1·9 mg. of tin, roughly 1·1 mg. was excreted in the urine and 0·7 mg. in the faeces. Probably the absorption of tin, as of heavy metals generally, is relatively greater the smaller the amount ingested. Some tin is always absorbed, and statements to the contrary are due to the limitations of the analytical methods used. It has been suggested that when tin salts in moderate amount are ingested over a long period changes may take place in the gastro-intestinal lining which favour the absorption of the metal (Salant, Rieger and Treuthardt 1914, 1918). Mention must also be made of the work of Misk (1923) who found comparatively large amounts of tin in the normal human body and considered that the body contains as much tin as it does zinc, presumably derived from canned food. Much of the tin so absorbed accumulates in the skin.

Goss (1917) refers to some unpublished experiments of Atkinson who found that in the electrolysis of pulped canned food with a mercury cathode only one-third to one-half of the tin present could be recovered, even after the food had been submitted to artificial gastric digestion. Goss separated the liquid from the solids in canned foods and showed that a large proportion of the tin in foods is combined in an insoluble form with food constituents. With fruits and berries most of the tin is in combination with the stones and seeds. On an average about two-thirds to three-quarters of the tin present is in the form of an insoluble protein compound which cannot be brought into solution by artificial digestion, but different foods show great variations. Bigelow (1916) also found that the greater part of the tin in canned beans and asparagus is in the solid part of the food, the proportion taken up by the solids increasing with the age of the sample. It appears highly probable that tin in combination with protein is not digested in the alimentary tract, or at any rate that a small residue of a highly resistant organic tin complex passes through unchanged. The effect of this on protein assimilation is relatively very small, but it is probably an important factor in the absorption and excretion of tin.

TOXIC ACTION OF TIN. Subsequent to Buchanan and Schryver's report, experiments on the effects of tin on animals have been described by Eckardt (1909), Eber (1910), Salant, Rieger and Treuthardt (1914), Goss (1917), Flinn and Inouye (1928) and Salant (1920). It has been found that tin salts in very large doses, for instance 1 g. every 6 or 10 days to rabbits, will kill the animal in one or two months with symptoms of stomach inflammation, degeneration of the liver and kidneys, and paralysis of the hind legs. Eber (1910) cites the case of a large dog which died after eating the remains of a number of cream cheeses together with their tin-foil coatings. In toxicological text-books the fatal dose of tin chloride for a dog is given as 4 to 6 g.

Cats have been given tin in the form of tartrate at the rate of 10, 20 and 30 mg. per kilogram of body weight daily for three months. No harmful effects were noticed with the lower doses, but a loss of weight occurred with 30 mg. per kilogram (Salant 1920). On the other hand, Lehmann in 1902 found that a cat of 1 kg. remained perfectly well and increased in weight on 40 mg. daily. He pointed out that a man of 75 kg. might possibly eat 420 mg. of tin daily, or 5·6 mg. per kilogram of body weight, if he lived exclusively on canned food, and considered that this diet would not be likely to cause tin poisoning.

It is difficult to find any instances in which poisoning, either acute or chronic, has been caused in human beings by tin in canned foods. According to Kenwood and Kerr (1929) a few cases of human poisoning have been recorded where tin to the amount of 15 to 20 gr. per lb. (2,000 to 3,000 p.p.m.) was found in the food, but in most of the cases ascribed to tin the evidence does not exclude the possibility of bacterial poisoning, which is a far more likely occurrence. At the same time it must be remembered that negative results obtained in experiments on animals, and on men, do not necessarily prove that tin has no remote toxic action. Some such action may exist and be undiscoverable by the methods of investigation at our disposal. Flinn and Inouye's suggestion referred to on page 409, that some metals considered to be non-toxic may possibly combine with constituents of the blood-cells or plasma and affect some of their functions, might apply particularly to tin which readily forms lakes with organic pigments. If limits for tin and other metals in food were made solely dependent on rigid experimental proof of injury to health, the combined effect on human nutrition might be disastrous. Whatever health significance is attached to the suggested limit of 2 gr. of tin per lb. in canned foods, it represents a liberal allowance for the manufacturer, especially in view of the great improvements in canning practice in the last 30 years, and at the same time, as far as can be seen, an adequate safeguard for the consumer.

THE DETERMINATION OF TIN IN FOOD

Schryver (1908) reviewed the possible methods of determining tin in canned foods and rejected those depending upon dry ashing, since the tin would be converted into an insoluble oxide which must be fused with alkali to bring it into solution. For a similar reason he avoided the use of nitric acid which might produce insoluble metastannic acid.

He adopted a method in which the food was digested with sulphuric acid and potassium sulphate as in the ordinary Kjeldahl process for nitrogen, with formation of soluble stannic sulphate. Tin in the resulting solution was either precipitated as sulphide and weighed as oxide or determined colorimetrically (p. 157). He also rejected volumetric methods of determining tin by reduction and iodimetric titration on the ground of probable interference by ferrous iron. Ferrous iron, however, is not oxidised in acid solution by iodine unless this is present in large excess and the mixture allowed to stand for some time ; on the contrary, ferric salts liberate iodine from hydriodic acid.

Schryver's sulphuric acid digestion method is long and troublesome and is not often used except for small samples of material. Wet oxidation with sulphuric and nitric acids is quicker, and tin is not as a rule precipitated as metastannic acid but remains in solution on careful dilution of the acid digest. From this it may either be precipitated as sulphide and weighed as oxide or it may be reduced to the stannous condition and titrated with iodine. If there happens to be any considerable precipitate of metastannic acid this can be brought into solution by diluting the strong acid liquid with a 5 per cent solution of tartaric acid and neutralising with sodium hydroxide, keeping the solution cool until alkaline (Allport and Skrimshire 1932).

GRAVIMETRIC METHOD. After destruction of organic matter by digestion with sulphuric and nitric acids the residual liquid is diluted and filtered if not clear. Adam and Horner (1937) collect any precipitate of calcium sulphate and silica that is formed, and fuse it in a silver crucible with sodium hydroxide to recover the small amount of adsorbed tin or metastannic acid which it may contain. The contents of the crucible are dissolved, acidified and added to the main quantity of liquid. Schryver, using a sulphuric acid digestion process with no nitric acid, found that any stannic sulphate retained by the precipitate of calcium sulphate, etc., could be extracted with hot concentrated hydrochloric acid. Unless the amount of precipitate is large, extraction with hot hydrochloric acid may be depended on to bring all the tin into solution, even when nitric acid has been used.

Precipitation with hydrogen sulphide can be carried out in strongly acid solution (Schryver) or after reduction to about 2 per cent of acid by addition of alkali (Willey 1924) or after neutralisation with ammonia (Adam and Horner 1937). The precipitate, containing all the tin as sulphide, is dissolved in sodium hydroxide solution, reprecipitated with acetic acid, dried, ignited and weighed as tin oxide. Adam and Horner digest the sulphide precipitate with yellow ammonium sulphide instead of sodium hydroxide. This is also the method adopted by the Association of Official Agricultural Chemists.

The gravimetric method, although long and troublesome, is accurate if care is taken to prevent contamination of the precipitate with silica, calcium salts or phosphates. Silica may cause trouble in the analysis of canned vegetables, and it may be necessary to heat the tin oxide precipitate with hydrofluoric acid in a platinum crucible.

Schreiber and Taber (1911) give an alkaline fusion method followed by gravimetric determination on the lines of Schryver's method.

REDUCTION OF STANNIC SALTS AND TITRATION WITH IODINE. This method, known as the Pearce-Low assay, is a standard method for tin ores and has been studied by many analysts. Papers by Hallett (1916), Clarke (1931), Evans (1931, 1944) and Okell and Lumsden (1935) should be consulted for a full discussion of the factors influencing the results and the precautions to be taken, both in the choice of a suitable reducing agent and in the titration of the resulting stannous chloride. Most of the work on this subject has been in connection with metallurgy and tin assaying, where special precautions must be taken against interference by other metals. In canned foods other metals are not often present in amounts sufficient to interfere with the accuracy of the titration.

Among the various reducing agents which have been used are metallic zinc, iron, lead, antimony, aluminium and nickel, and also hypophosphorous acid. Stannous chloride is extremely readily oxidised by air, especially in strong hydrochloric acid solution and in presence of hydriodic acid, both of which appear to act as oxidation catalysts. The titration must therefore be carried out in an atmosphere of carbon dioxide with rigid exclusion of air. Even the oxygen dissolved in standard iodine solution may cause a serious error when titrating small amounts of tin with 0·01N solutions. Evans (1927) and Willey (1924) have described special arrangements for ensuring complete absence of air during reduction and titration, and Okell and Lumsden (1935) a method for preparing air-free standard iodine solution.

Of the reducing agents, zinc and aluminium have the advantage that the solution remains colourless, while iron, and still more nickel, colour it green and tend to obscure the end-point. Zinc and aluminium have, however, the disadvantage that they both reduce tin salts to spongy metallic tin, which must be dissolved before titration without any reducing agent being present. Antimony in powder reduces stannic to stannous salts and does not itself interfere with the iodine titration if enough hydrochloric acid is present, except in so far as the suspended powder obscures the end-point. Lubatti (1923) obtained good results by using large quantities (10 g.) of antimony in fragments. Metallic antimony is, however, often impure and may contain traces of tin, and its use as a reducing agent is not to be recommended. Occasionally antimony may be present with tin in food, as, for instance, in foil-wrapped cheeses or in foods which have, before canning, been in contact with soft enamels containing antimony. Antimony salts are more easily reduced than those of tin, and any metal which will reduce tin will also reduce antimony to the metallic state. The presence of reduced antimony does not affect appreciably the determination of tin. It can be dissolved by heating for some time with hydrochloric acid (1 : 2). Solutions of antimony trioxide do not react with iodine in strongly acid solution if a large excess of potassium iodide is present: antimony can be determined after titration of tin by making the solution alkaline with sodium bicarbonate and titrating with standard iodine solution.

Nickel has been used successfully by Hallett (1916) and iron by some workers (Clarke 1923). They have the advantage that they do not precipitate metallic tin from solution, but the solution after reduction is more or less coloured. Lead is one of the best metallic reducing agents. It does not precipitate metallic tin, the solution is colourless and the residual lead need not be removed before titration ; also it can readily be obtained pure and free from other metals. Clarke (1931) boils the solution of tin, containing 50 ml. of concentrated hydrochloric acid in 250 ml., for 1½ hours with lead foil in an atmosphere of carbon dioxide and titrates with standard iodine solution. Separation of crystals of lead chloride can be prevented by the addition of salt and the presence of a large amount of hydrochloric acid (Powell 1918). Evans (1927) prefers to remove residual lead and describes an arrangement for separating the reduced tin solution from lead without access of air. The titration is carried out in presence of oxalic acid. Copper interferes since it is precipitated by lead and takes tin down with it as the compound $Cu_{10}Sn_3$ (Clarke 1931).

Aluminium is used as a reducing agent by Owe (1926) and by Lunde and Mathieson (1934). It is also used in the tentative method of the Association of Official Agricultural Chemists (1945). Too much aluminium should be avoided since the metal is liable to contain silica, and silica in the solution to be titrated may absorb a small amount of iodine. Owe treats 40 g. of food with 25 ml. of sulphuric acid, and nitric acid until completely oxidised. Residual nitric acid is removed by heating with 25 ml. of saturated ammonium oxalate solution, the liquid is diluted to 300 ml., 25 ml. of concentrated hydrochloric acid is added, a current of carbon dioxide is led into the flask, and the tin is reduced with 0·4 g. of aluminium turnings. The flask contents are heated until the metallic tin which separates is re-dissolved, 25 ml. of 0·02N iodine solution are added and the excess of iodine titrated with thiosulphate, using starch as indicator. 1 ml.

of 0·02N iodine solution is equivalent to 1·23 mg. of tin. Copper, if present, interferes with the titration and causes the apparent amount of tin to be too high.

Evans (1931, 1944) recommends hypophosphorous acid as the best reducing agent for tin. In strongly acid solution it reduces tin salts to the stannous condition, but on dilution to one-fifth of the acid strength and cooling, hypophosphorous acid does not react with iodine. Tin is reduced by boiling for 15 minutes in a carbon dioxide atmosphere with a solution of sodium hypophosphite in diluted hydrochloric acid (1 : 1) and a little mercuric chloride or mercuric cyanide as catalyst. After cooling, the solution is diluted with boiled water containing citric acid, starch and potassium iodide and titrated with standard iodine solution. Hypophosphorous acid reduces quinquevalent salts of antimony to tervalent salts but not to metal. It will reduce arsenic compounds to metallic arsenic.

In all these methods nitric acid must be absent. It can be removed from the acid residue after wet oxidation, by heating with saturated ammonium oxalate solution. Lunde and Mathieson (1934) find that one treatment is not always enough and that it must be repeated to ensure absence of all traces of nitrogen acids.

As already mentioned, it is not usually necessary in examining canned foods to undertake a preliminary separation of tin from traces of other metals before titration. Many analysts prefer to separate tin first as sulphide (Willey 1924) or reduce it to metal with zinc and filter the metallic tin from the solution before re-dissolving it in hydrochloric acid for titration (Clarke 1923), but this would seem to be necessary only in exceptional circumstances. Possible errors due to oxidation of stannous chloride can be minimised by standardising the iodine solution with metallic tin under conditions precisely similar to those of the actual determination ; if, however, care is taken to exclude air the theoretical factor for iodine solution can safely be used.

COLORIMETRIC DETERMINATION. There are three colorimetric methods for tin, one of them a direct method with toluene 3:4-dithiol and the other two indirect methods depending on the reducing action of stannous chloride.

With Toluene 3:4-Dithiol. Dimercapto-benzenes (benzene dithiols) form red precipitates with tin, the constitution of which is not yet determined. The reaction has the advantage of being almost specific for tin. Clark (1936, 1937) finds that toluene 3:4-dithiol, $CH_3C_6H_3(SH)_2$, is the best reagent for the purpose. It can be obtained commercially under the name 'dithiol' or made by reducing toluene 3:4-disulphonyl chloride with tin and hydrochloric acid (Mills and Clark 1936). It is a colourless crystalline compound melting at 35°.

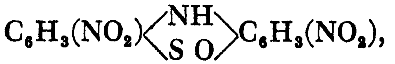
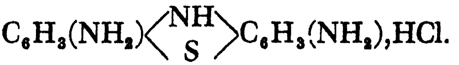
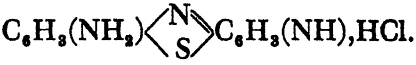
Dithiol gives a bright red precipitate with stannous tin and a brick-red precipitate with bismuth. Also it gives black precipitates with copper, nickel and cobalt. With other metals the colours are various shades of yellow which do not interfere with the red colour due to tin, provided that the dithiol is in excess. Phosphates interfere with the reaction if present in the liquid in concentration greater than 0·05 per cent. With many foods therefore it is necessary first to separate tin as sulphide.

Since the red compound is a precipitate it must be held in suspension by a colloid such as agar-agar for colour comparison and is best examined by reflected light rather than by transmitted light. The red precipitate is given only by stannous tin, and a small quantity of thioglycollic acid is therefore added to ensure reduction of all the tin present to the stannous condition. This does not cause interference by iron since the solution is acid and iron gives a purple colour with thioglycollic acid in alkaline solution only (p. 263). Nitrous acid must be absent as it gives a red colour with thioglycollic acid.

The acid solution containing tin after addition of thioglycollic acid is diluted until it contains not more than 60 p.p.m. of tin. 5 ml. of the solution are placed in a graduated test-tube, 1 ml. of concentrated hydrochloric acid and 1 ml. of a warm agar-agar jelly are added and the solution is heated to boiling until the agar-agar is dissolved.

The tube is cooled and treated with 2 ml. of a solution containing 0·2 per cent of dithiol, 0·3 to 0·5 per cent of thioglycollic acid and 1 per cent of sodium hydroxide. The volume of the solution is made up to 10 ml. with water, the tube is immersed in a boiling water bath for one minute and the colour compared with standards. Clark gives a graph by means of which the amount of tin can be ascertained by the colour units of Lovibond's tintometer.

This method has been applied to the determination of tin in foods by de Giacomi (1940). He uses a sulphuric acid–potassium sulphate wet oxidation method for destroying organic matter, on the ground that nitric acid may cause separation of insoluble metastannic acid. Tin is first separated as sulphide to avoid the interference of phosphates. By keeping the quantity of tin in the test solution below 30 p.p.m. he avoids the necessity of using agar-agar. Law (1942) separates tin first as stannic bromide in preference to sulphide. The material is charred in a silica dish and tin distilled as stannic bromide, $SnBr_4$, by heating with sulphuric and hydrobromic acids at 220° in a current of carbon dioxide. A special still with ground-in glass connections is used. Stone (1941) applies the dithiol method to malt beverages, and Dickinson (1944) to canned foods, without removal of phosphate, the amount present in the final solution being too small to interfere appreciably with the determination. They use a dry-ashing method and reduce the tin to metal by fusion with cyanide and alkaline carbonate. The melt containing metallic tin is then dissolved in hydrochloric acid and treated with a solution of dithiol containing thioglycollic acid.

With Dinitro-diphenylamine-sulphoxide. Dinitro-diphenylamine-sulphoxide,  $C_6H_3(NO_2)\left\langle\begin{smallmatrix}NH\\ S\ O\end{smallmatrix}\right\rangle C_6H_3(NO_2)$, is reduced by stannous chloride to the hydrochloride of diaminothiodiphenylamine,  $C_6H_3(NH_2)\left\langle\begin{smallmatrix}NH\\ S\end{smallmatrix}\right\rangle C_6H_3(NH_2),HCl$. This body on oxidation with ferric chloride loses two hydrogen atoms and gives Lauth's violet,  $C_6H_3(NH_2)\left\langle\begin{smallmatrix}N\\ S\end{smallmatrix}\right\rangle C_6H_3(NH),HCl$.

A colorimetric method based on this reaction was developed by Schryver in a report on canned foods to the Local Government Board (Buchanan and Schryver 1908). Tin is first separated as sulphide which is dissolved in concentrated hydrochloric acid and the solution filtered. The stannic chloride is then reduced to stannous chloride by heating with zinc foil in an atmosphere of carbon dioxide, and 2 ml. of a solution of 0·2 g. of dinitro-diphenylamine-sulphoxide in 100 ml. of 0·1N sodium hydroxide added. After further boiling for a minute or two, the solution is diluted to 100 ml. with water and filtered. It turns violet during filtration, and the full depth of colour is reached on addition of a drop of dilute ferric chloride solution.

This method is capable of giving good results but is not now often used as it is troublesome to operate and requires much practice. Also it is not at all certain that the presence of other reducing agents besides stannous chloride may not vitiate the results.

Molybdenum Blue Method. Munro (1927) found that the formation of molybdenum blue, Mo_2O_5, $4MoO_3$, by reduction of ammonium molybdate with stannous chloride (cf. p. 198) could be used to detect the presence of less than 1 p.p.m. of tin in a solution. Strafford (1933) reduces tin to the stannous condition with aluminium in an atmosphere of carbon dioxide, adds an acid molybdate solution after cooling, allows the liquid to stand for one minute and then extracts the molybdenum blue with 10 ml. of amyl alcohol. The blue amyl alcohol solution is slightly turbid but may be cleared by adding 1 ml. of ethyl alcohol. The colour is then measured in a Lovibond tintometer, the blue units being taken as a measure of the tin.

The test is more satisfactory in presence of a phosphate. Molybdenum blue is unstable on dilution, but if phosphates or arsenates are added a reduced phosphomolybdate or arsenomolybdate is formed and the stability of the blue is greatly increased. Davies (1932) applied the test in this form to the detection of tin in cheese. The cheese is moistened with water and one drop of a 0·5 per cent solution of ammonium molybdate in normal hydrochloric acid added, followed by a drop of 5 per cent sodium phosphate solution. If tin is present an intense blue colour develops.

The molybdenum blue reaction is used for the determination of phosphates and arsenates (p. 198). It has been applied to the determination of copper by first reducing the copper salt to metal and then allowing the metallic copper to reduce a solution of phosphomolybdic acid (p. 46). The reaction can be used in a similar manner for the determination of stannous salts or of metallic tin, in quantities down to 0·1 mg. (Hüttig 1923, Feigl 1923). It has, however, the disadvantage that it is not specific for tin and may be brought about by other reducing agents.

In the so-called 'cacothelin' reaction the reagent is nitro-bruciquinone hydrate, made by treating brucine with nitric acid. It is reduced to a violet dye by stannous tin and also by lower oxides of molybdenum, tungsten and uranium (Beck 1938) and by many other substances (Rosenthaler 1938). The test has not been applied quantitatively.

SPECTROGRAPHIC METHOD. Kent (1942) has determined tin in cereal products by a spectrographic method, an outline of which is given on page 97.

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5

[ARSENIC]

SO FAR as is known, arsenic[1] plays no part in natural biological processes. Estimates differ as to the amount usually present in the body. According to Steinbrück (1938) it may normally be as much as 0·3 p.p.m. The blood is said to contain 0·6 p.p.m. (Guthmann and Grass 1932), which would imply about 3 mg. in the total quantity of blood. The daily excretion in the urine has been given as 0·008 to 0·150 mg. per litre : Mattice and Weisman (1937) give a still wider range up to 0·85 mg. per litre (cf. also Cox 1925). The hair and nails frequently contain more arsenic than any other part of the body. Hair generally shows 0·3 to 0·7 p.p.m. and nails 1·5 to 4 p.p.m. (Szép 1940), but after large doses they contain much more and sometimes furnish important evidence in cases of poisoning. After acute arsenical polyneuritis the nails may contain from 20 to 120 p.p.m. (van Itallie 1932). The arsenic normally found in the body may be derived mainly from fish (p. 171) or from inhaled dust and coal smoke.

COMPARATIVE TOXICITY FOR MAN AND ANIMALS. The minimum fatal dose of arsenious oxide for a man is given as 0·06 to 0·18 g. or about 0·8 to 2·4 mg. per kilogram. For the lower warm-blooded animals it is considerably greater, of the order of about 15 mg. per kilogram body weight and for insects still more, but animals differ greatly in susceptibility. According to Willberg (1913) lower animals tolerate arsenic better than human beings owing to their nervous system not being so highly developed.

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[1] Except where otherwise indicated the word ' arsenic ' is used to denote arsenic trioxide or arsenious oxide As_2O_3.

Chickens are stated to be particularly resistant (Whitehead 1934). Franke and Moxon (1937) find that 50 p.p.m. of arsenic trioxide in the food of rats is slightly toxic. This corresponds to 0·5 mg. daily or about 2 mg. per kilogram of body weight.

Wiebe (1930) found that several species of fresh-water fish could withstand arsenious oxide up to 7 p.p.m. in water for at least 6 days, and in some experiments a much higher degree of resistance has been observed. Trout are not affected by 10 p.p.m. of arsenic in hard water. Weigelt (1900) gives a table showing the effect on young salmon, trout and char of solutions containing from 10 to 390 p.p.m. of arsenic with exposures varying from 40 minutes to 4 hours. Arsenic is not nearly so poisonous to fish as copper (p. 13). Surber (1931) found that excessive growth of weeds in ponds could be destroyed by 1 to 1·8 p.p.m. of arsenic in the form of sodium arsenite without harming fish. If more than 2 p.p.m. is used there is a danger of killing the fauna which serve as fish food. 2 p.p.m. of arsenic in water will kill May-fly nymphs and fresh-water shrimps and their larvae, but a higher concentration is required to kill other aquatic insect life. Protozoa survive 3·5 p.p.m. Some insects such as dragon-fly nymphs will stand as much as 10 to 30 p.p.m. of arsenic in water.

At high levels of intake arsenates are less toxic than arsenites. Franke and Moxon (1937) found that when injected intraperitoneally into rats, the minimum fatal dose in the form of sodium arsenite was about 4·5 mg. per kilogram body weight as against 16 mg. per kilogram for arsenic in the form of sodium arsenate. It is doubtful, however, whether such distinction can be made at low levels of intake in food. The toxic action of arsenious oxide has been shown to be due to its combination with thiol compounds in the body with formation of thio-arsenites. It interferes, therefore, with enzyme systems, chiefly the pyruvate oxidase system, which depend upon these thiols for their operation (Peters, Stocken and Thompson 1945, Stocken and Thompson 1946). Arsenic acid and arsenates do not react with thiols, and are in consequence not acutely toxic until reduced in the body to arsenious acid or arsenites. Arsenates are in general eliminated by the kidneys much more readily than arsenites. It would be expected, therefore, that arsenates will be less toxic at high levels of intake than arsenites, but that at low levels reduction to arsenite may be sufficiently rapid and complete to eliminate any difference in toxicity. According to Thuret (1939) arsenic acid is reduced to arsenious acid in the liver, probably by glutathione, and it is possible that reduction takes place also in the alimentary tract. The relative insolubility of some arsenic compounds in the digestive tract may also affect toxicity. Insoluble lead arsenate has been stated by Cardiff (1940) and Webster (1941) to be relatively less toxic to human beings on this account, although it is an effective insecticide (p. 79).

ARSENIC CONTAMINATION OF FOOD. In 1900 there was a severe outbreak of arsenic poisoning in Lancashire and Staffordshire due to beer made with arsenical glucose. The total number of cases was estimated at over 6,000, including at least 70 deaths and probably many more in which the cause of death could not be ascribed with certainty to arsenic. There is reason to believe that arsenic poisoning from this cause had been prevalent in the Manchester district for many years before 1900, but that it had been mistaken for alcoholic neuritis. Samples of the glucose in question were found to contain several hundred parts of arsenic per million, and the beer from 5 to 15 p.p.m. with occasional samples as high as 20 p.p.m.

The outbreak was quickly followed by the appointment of a Royal Commission, under the chairmanship of Lord Kelvin, to investigate the whole question. The report of the Commission and minutes of evidence, published in two volumes by H.M. Stationery Office in 1903, contain a full record of the inquiry and a number of appendices. They represent a complete and detailed survey of practically the whole field of arsenic contamination of foods, and little more can be added after nearly fifty years except in the matter of improvements in analytical technique.

The recommendation of the Commission that the limits for arsenious oxide should be $\frac{1}{100}$ gr. per lb. for solid foods (1·4 p.p.m.) and $\frac{1}{100}$ gr. per gallon for liquid foods (0·14 p.p.m.) has in only one instance been given statutory force but has nevertheless been generally adopted. In making this recommendation the Commission did not say whether the term 'liquid' was to be held to include syrups, but it may be noted that, on page 50 of the report, golden syrup is referred to as a solid. Presumably therefore the word 'liquid' is intended to include only beverages and foods or food ingredients of similar consistency. Sauces such as mayonnaise and tomato ketchup would accordingly be classed as solids.

The difference in limits for arsenic suggested for solids and liquids is interesting. We are not told whether this differentiation was made because liquid foods contain comparatively little solid matter, or because the consumption of liquids may be, weight for weight, much greater than that of solids, or because absorption of toxic substances in the alimentary tract is greater from liquids than from solids: probably all three points were taken into account. The question of relative extent of absorption is of great importance not only with arsenic but with lead and other metals. In fact, it is only by assuming a much higher degree of absorption from liquids than from solids that many of the apparent contradictions in metallic poisoning can be explained (p. 71).

The Commission were not able to say with certainty how much arsenic is necessary to produce symptoms of chronic poisoning. They referred to the fact that tolerance is sometimes shown to doses as high as 20 mg. ($\frac{3}{10}$ gr.) three times daily while on the other hand there are many examples of high individual susceptibility. A dose of 6 mg. ($\frac{1}{10}$ gr.) daily for 3 weeks has produced paralysis and a single dose of 1·3 mg. ($\frac{1}{50}$ gr.) is alleged to have been followed by symptoms of poisoning. It would seem therefore that even with the limits suggested by the Commission the margin of safety is not excessive, and it has been the practice among those responsible for the administration of the Food and Drugs Act not to make concessions save in exceptional circumstances. The Commission in their report said that 'it would be unwise to express an opinion that any quantity of arsenic, however small, is to be regarded as admissible in any article of food' and suggested that 'it should be the aim of the food manufacturer to exclude arsenic altogether from his products'. It should be noted particularly that the Commission's limits were intended to be applied not only to food as consumed but also to the individual ingredients of foods before they are mixed or diluted for consumption.

In times of emergency, during the years 1915–18 and 1940–6, with restrictions on the choice of raw materials, it has sometimes proved difficult or impossible for manufacturers to keep within the Royal Commission's limits, and attempts have been made to discount these limits as antiquated and unnecessarily stringent. There are undoubtedly a few cases in which some relaxation may be allowable, but very few. Until more precise knowledge is available of the cumulative effect of repeated minute doses of arsenic in food, it is clear that on return to more normal conditions the highest possible standard of purity in this respect must be insisted upon.

From a study of the Commission's report, which covered a very wide range of foods, it is evident that arsenic contamination of food at that time arose chiefly from the use of arsenical pyrites in making sulphuric acid. Sulphuric acid enters at some stage into the manufacture of many foods and food ingredients, such as sugars used in brewing and confectionery, citric, tartaric and phosphoric acids and their salts, vinegar, glycerine, food colours and pigments, yeast, caramel. Acid calcium phosphate, for instance, prepared from bone ash with sulphuric acid, has been found to contain as much as 400 to 600 p.p.m. of arsenic (MacFadden 1917). Since much of the sulphuric acid of commerce is now made by the contact process there is not so much risk of arsenic gaining access to food from this source.

Coke produced from coal containing arsenical pyrites is often highly charged with arsenic, and malt dried with coke fumes may become contaminated. Several cases of poisoning which occurred at Halifax in 1902 were traced to beer made from malt containing about 7 p.p.m. As a result of this the use of gas coke for drying malt was discontinued in favour of non-arsenical Welsh anthracite. The possibility that other foods (e.g. vegetables), which have been dried over coke fumes, may contain arsenic should always be borne in mind. Grain dried over coke in farm driers has been found to be only slightly contaminated (Jones and Dawson 1945).

Food may collect traces of arsenic from several sources other than sulphuric acid and coke fumes. Some of these were referred to in the Commission's report and others are of more recent discovery. The most important are those concerned, either directly or remotely, with the use of arsenical insecticides in agriculture.

At one time it was thought that serious contamination of food might result from the use of arsenical war gases, but no instances of this have been reported.

ARSENICAL INSECTICIDES AND FOOD CONTAMINATION. Lead arsenate is widely used for spraying apples, pears and other fruit as a protection against several different species of caterpillar, particularly that of the codlin moth (*Carpocapsa pomonella*). This moth is very destructive in the United States and lead arsenate spraying is practised there on a large scale. Some years ago apples imported into this country were found to contain excessive amounts of arsenic in the form of adhering spray residue, in several instances as much as 1 to 2 mg. being found on one apple.

Lead arsenate may be either the acid arsenate, $PbHAsO_4$, containing about 30 per cent of As_2O_5, or the basic arsenate $Pb_3(AsO_4)_2$ with about 20 per cent of As_2O_5. The acid arsenate is the one most generally used (p. 79). A number of different ' spreaders ' have been used to make the insoluble lead arsenate cover as much surface as possible and stay on the fruit for a long time. Lead arsenate is partially converted by weathering into a basic arsenate, $Pb_4PbOH(AsO_4)_3$, with liberation of soluble arsenic acid, so that after a time the lead : arsenic ratio changes from about 2 : 1 to about 3·5 : 1

(Fahey and Rusk 1939). Thus arsenic in sprayed apples decreases in amount more quickly than lead.

Lead arsenate spray residue is removed from fruit commercially by dipping it for about a minute into 1 per cent hydrochloric acid followed by washing with water. This treatment is now almost universally applied, and residual arsenic on fruit imported into this country seldom exceeds the limit of 1·4 p.p.m. In the United States the limit for fruit and vegetables in interstate commerce is 0·025 gr. per lb. (3·6 p.p.m.). Calcium, manganese, copper and zinc arsenates have also been used for fruit spraying, but manganese and zinc arsenates are liable to cause burning of foliage. Some commercial acid lead arsenates are also liable to burn foliage unless the amount of soluble arsenic in the preparation is kept below 0·25 per cent (Swingle 1929).

Wine and cider may contain arsenic derived from sprayed fruit, but the greater part of it remains in the lees or is removed by yeast during fermentation. Wine yeasts may contain as much as 150 to 180 p.p.m. of arsenic in the dry matter (von der Heide 1922). According to Bosselmann and Koch (1923) yeast produces small quantities of hydrogen sulphide ; this precipitates arsenic as colloidal sulphide, which is then adsorbed by the yeast. Imported bakers' yeast has been found to contain up to 17 p.p.m. of arsenic (*Ann. Rept. C.M.O.* 1931). Sweet musts and unfermented fruit juices are more liable than wines to retain spray arsenic.

Vegetables, especially cabbage and celery, are sometimes dusted with lead arsenate and may retain excessive amounts. With potatoes, spraying of the haulm with lead arsenate does not lead to contamination of the tubers (Wührer 1938).

The soil of orchards in which lead arsenate spraying is practised may eventually become charged with arsenic to the extent of several hundred parts per million. Such soils may be toxic, especially to leguminous crops, the toxicity depending largely upon the amount of soluble arsenic, which is usually about one-tenth of the total arsenic in the soil (Greaves 1934). The proportion of soluble arsenic can be reduced by treatment of the soil with ferric sulphate. Practically all the arsenic is retained in the top layers of soil and does not penetrate to the roots of orchard trees. The amount taken up by plants from the soil, even when it is highly charged, is negligible. In general, arsenates in soil are much less toxic to plants than arsenites. Normal soils may contain from 0·3 to 38 p.p.m. of arsenic (Williams and Whetstone 1940).

Spraying of fruit trees with arsenicals may have a serious effect on bees. In certain districts of the United States losses of half the bee colonies from this cause have been recorded (Webster and Crews 1934), and in this country also spraying at the wrong time is often responsible for serious losses. In bees killed by arsenic spraying Lockemann (1935) found 1 part of arsenic in about 45,000 parts by weight of the dead bees, and Graham (1942) gives a figure of the same order. Assuming that a bee weighs about 100 mg. the lethal dose is therefore about 2 μg. The honey itself does not contain significant amounts.

On several occasions liquid arsenical sheep dip and weed killer have been packed for transit in the same compartment as sugar and other foods, and these have become seriously affected by leakage.

A curious instance of arsenic gaining access to food from an unsuspected source was that of cocoa in 1922. The cocoa had been treated with potassium carbonate to make it ‘ soluble ’, and it was suggested that the potassium carbonate had probably been prepared from residues from the washing of wool from sheep which had been through an arsenical dip. Contamination of baking powder containing alkaline carbonate has been traced to a similar source (Goy and Köhler 1925).

Gelatin made from hides, tanning waste, etc., is often contaminated owing to the use of arsenious oxide and sulphide in the preparation of the hides (Köpke 1911). In New South Wales in 1931 the limit for arsenic in gelatin was placed at 4·2 p.p.m. In this country a limit of 1·4 p.p.m. ($\frac{1}{100}$ gr. per lb.) has been prescribed under the Edible Gelatin (Control) Order, 1947.

ARSENIC IN FOOD COLOURS AND PIGMENTS, PRESERVATIVES, ETC. The presence of arsenic in food colours was investigated in 1928 by a joint committee representing the Society of Public Analysts and the Association of British Chemical Manufacturers. They referred in their report to the difficulty of preparing synthetic colours with an arsenic content as low as 1·4 p.p.m. and suggested that in view of the small amount of colour added to foods the Royal Commission's limit should be relaxed. They recommended limits of 5 p.p.m. of arsenious oxide for pure (straight) colours, 3 p.p.m. for colours diluted with not more than twice their weight of diluent and 1·4 p.p.m. for colours diluted to a greater extent. The usual diluents are sodium sulphate, sodium chloride and dextrin. Some synthetic dyes may contain comparatively large amounts of arsenic, notably Orange II (Stern 1928). The bad reputation of Orange II for toxicity may have been due to its frequent contamination with arsenic and not to any specific property of the dye itself.

Coloured glazed papers used for wrapping chocolate and other confectionery used often to be highly charged with arsenic (Stern 1928). In particular, violet and magenta papers were coloured with lakes precipitated from basic dyes with sodium arsenite. Some of these lakes may contain as much as 40 or 50 per cent of arsenious oxide. A coloured paper widely used some years ago for wrapping a well-known brand of chocolate contained 0·5 to 0·6 per cent of arsenic. In the absence of any regulations on the subject several manufacturers have provisionally adopted a limit of 10 p.p.m. for the arsenic content of wrapping papers.

Pigments such as oxide of iron (Armenian bole) and Prussian blue are liable to contain arsenic derived from arsenical sulphuric acid. Since these pigments are occasionally added direct to foods in fairly large quantity (pp. 254, 256) the arsenic content should certainly not exceed the limits suggested above for synthetic food colours. Another possible source of arsenic contamination is shellac, sometimes used for glazing confectionery and for varnishing vats and casks in breweries. The colour of shellac used to be 'improved' by the addition of orpiment, or yellow arsenic sulphide, some samples containing as much as 0·15 to 0·2 per cent (Smith 1912). The colouring of food with any compound of arsenic is specifically prohibited under the Preservatives Regulations 1925–7. Arsenic is sometimes used as an ingredient of cheap hardware enamels, but there have been no instances in recent years of its occurrence in the enamel of hollow-ware sold for kitchen use.

Demerara sugar is coloured with tin chloride which probably forms a tin lake with the natural colouring matter of the sugar-cane (p. 146). Commercial tin may contain from 0·03 to 0·05 per cent of arsenic, but since the amount of tin in Demerara sugar is not usually more than 150 p.p.m. the quantity of arsenic finding its way into the sugar from this source is negligible. In canned foods the likelihood of contamination is also small.

Boric acid may contain arsenic up to several hundred parts per million, partly natural to native borax and partly derived from sulphuric acid during manufacture. Such borax, if added to food as a preservative, may contribute an appreciable amount of arsenic.

It has been suggested that the use of arsenic for fattening poultry and also the intravenous injection of arsenical drugs (neo-salvarsan) as a treatment for entero-hepatitis in turkeys (*Ann. Rept. C.M.O.* 1933) may possibly lead to contamination of the flesh, but this seems unlikely, although the livers of turkeys so treated may contain more than $\frac{1}{100}$ gr. per lb.

A recent instance of serious arsenic contamination of food was the accidental use of white arsenic instead of starch for dusting confectionery at Burslem (*Ann. Rept. C.M.O.* 1930). Many children were affected, but fortunately there were no deaths, possibly because the amount of arsenic was so large that the children all promptly vomited.

ARSENIC IN FISH. The Swedish Arsenic Commission (Cox 1925) found that sea-fish contain arsenic. Plaice in particular showed 3 to 4 p.p.m. This may be due to their feeding on shell-fish and crustaceans which contain notable quantities (*Fishery Investigations* 1924, Chapman 1926). Chapman found 3 to 10 p.p.m. in oysters and as much as 120 p.p.m. in other shell-fish, particularly mussels. In crustaceans also the amounts were very high, up to 170 p.p.m. in prawns. The arsenic is all in organic combination and cannot be determined by the Marsh-Berzelius method unless organic matter has first been destroyed. After digestion *in vitro* with pepsin and trypsin it still cannot be detected by this method although it is in solution. It is excreted in urine, also in organic combination. Chapman found that after a meal of lobster containing 70 p.p.m. of arsenic the urine showed as much as 11 p.p.m. Feeding experiments on rats with shrimps containing 170 p.p.m. have shown no ill effects (Coulson and others 1934), and it would seem that the metabolism of arsenic in the form in which it occurs in crustaceans may be different from that of inorganic arsenic. Sea-water contains only 0·006 to 0·03 p.p.m. of arsenic, but estuary water, contaminated with industrial effluents, may contain from 0·14 to 1 p.p.m., the average found by Chapman for the Thames estuary being 0·33 p.p.m.

Shell-fish circulate relatively enormous quantities of water through their gills : it has been estimated that one mussel can pass as much as 10 gallons of water through its siphon tubes in 24 hours. Clearly, therefore, any tendency on the part of the tissues to adsorb arsenic or heavy metals from water may result in a relatively large accumulation of the adsorbed substance. One need not assume that arsenic or heavy metals play any part in the physiology of shell-fish. Adsorption and accumulation may be unavoidable, and the organism may have developed means of making the adsorbed metal innocuous. Shell-fish of the same species from different localities show wide variations in arsenic content.

Fish and crustaceans from fresh water do not contain abnormal amounts of arsenic (Chapman 1926) but may take it up if the water becomes contaminated (Ellis 1934).

THE DETERMINATION OF ARSENIC IN FOOD

The determination of arsenic in food has probably received more attention than that of any other trace element owing to its great toxicity and to the many ways in which it may gain access to food. Since the outbreak of arsenical poisoning in Lancashire in 1900 and the report of the Royal Commission of 1901–3 the examination of foods and food ingredients for arsenic has become an indispensable routine operation.

REINSCH TEST. At the time of the Lancashire outbreak the Reinsch test was widely used. A relatively large quantity, several hundred millilitres, of beer was strongly acidified with hydrochloric acid and heated for about an hour with a small piece of copper gauze. The film of metallic arsenic which formed on the gauze was volatilised by heat in a small glass tube and the crystals of arsenious oxide in the sublimate identified under the microscope. This test was stated to be capable of detecting with ease one part of arsenic in one million of beer and possibly still smaller amounts. As ordinarily used, however, in toxicological work the Reinsch test is credited with a delicacy not greater than 1 in 250,000.

Griffiths (1941) has described a modification of this test which is sensitive to 0·001 mg. of arsenic and which can be used as a sorting test for food samples after wet oxidation with sulphuric and nitric acids. The stains on copper foil are compared with standards deposited on pieces of copper foil of the same dimensions, cut from the same sheet and cleaned in exactly the same way so that the surfaces are closely similar.

The chemistry of the Reinsch test has been investigated by Evans (1923), who showed that arsenic is not deposited unless a halide acid is present and further that cupric salts inhibit the reaction, so that it is essential to ensure that the cuprous chloride in the liquid cannot become oxidised in any way, even slightly. If much copper is present in solution it can be reduced by adding sodium hypophosphite (Clarke and Evans 1929). If nitric acid is present it should be removed by fuming down with sulphuric acid and treatment with ammonium oxalate, as it is somewhat resistant to the reducing action of sodium hypophosphite.

Antimony, bismuth, selenium and mercury are also deposited on copper from hydrochloric acid solution.

CONVERSION OF ARSENIC COMPOUNDS TO ARSINE. Most methods of determining arsenic in foods are based upon its reduction to arsenic hydride (arsine) by nascent hydrogen, generated either from zinc and acid or electrolytically. Three distinct processes are involved in the production of arsine—the reduction of arsenic acid to arsenious acid, of arsenious acid to elemental arsenic, and of elemental arsenic to arsine. The speed and completeness with which each of these processes takes place depend upon the conditions under which the reduction is carried out. The first and third stages of the reduction are effected more slowly and with greater difficulty than the intermediate stage.

HYDROGEN OVERVOLTAGE. It is generally accepted that the chief factor in the reduction of arsenic acid to arsenious acid is the hydrogen overvoltage, or supertension, of the metal employed, that is to say, the relative energy required in a given electrolyte to liberate hydrogen gas from the surface of a metal electrode. In an electrolytic cell the reaction involved may be represented as $2H^+ \rightarrow H_2$ or, in accordance with more recent views involving the union between a hydrogen ion and an electron on the cathode to give atomic hydrogen, as $H^+ + \varepsilon \rightarrow H$ or perhaps $H_3O^+ + \varepsilon \rightarrow H + H_2O$. This reaction is much slower for some metals, those with high overvoltage, than for others. Positive hydrogen ions therefore may reach a high concentration on the surface of the metal and exert an intense reducing effect before they are released as gas, and will raise the electrical potential of the surface layer at the cathode by a corresponding amount (hydrogen polarisation).

The hydrogen overvoltage of the common metals was first measured by Caspari (1899), whose figures are given here:

somewhat similar results, varying with the conditions of experiment, were obtained by Tafel (1905) and by Newbery (1916).

Hg	Zn	Pb	Sn	Cd	Cu	Ni	Ag	Pt (smooth)	Pt (platinised)
0·78	0·70	0·64	0·53	0·48	0·23	0·21	0·15	0·09	0·005 volts

The reasons for the difference in overvoltage shown by different metals have not been fully explained, in spite of the large amount of work done on the problem in recent years (Glasstone 1937, Bowden and Agar 1938). Overvoltage depends on a number of factors which are difficult to resolve experimentally. The speed of the reaction $H^+ + \varepsilon \rightarrow H$, that is to say the readiness with which the cathode metal gives up an electron to a hydrogen ion, is apparently a specific property of the metal itself, but since the overvoltage depends upon the concentration of hydrogen ions at the surface of the cathode it is affected by the amount of metallic surface available, i.e. whether the electrode is rough or smooth. If the electrode surface is relatively small and the reaction $H^+ + \varepsilon \rightarrow H$ is slow, as with mercury, saturation with hydrogen is quickly reached and maintained and the overvoltage and reducing power are high. If the cathode surface is relatively large and the reaction $H^+ + \varepsilon \rightarrow H$ is rapid, as with platinised platinum, the surface never becomes saturated with hydrogen and the overvoltage and reducing power are low. When the surface is saturated with hydrogen ions the hydrogen overvoltage varies as the logarithm of the current density. When it is unsaturated, the hydrogen overvoltage varies directly with the current density. Overvoltage thus depends upon the combined working of a hydrogen absorption mechanism for unsaturated surfaces and a discharge mechanism for saturated surfaces.

ZINC AND ACID METHOD. In the method recommended in 1902 by the Joint Committee of the Society of Chemical Industry and the Society of Public Analysts (*Arsenic Commission Report*, vol. II, Appendix 20, p. 206) the sample of food, sometimes after destruction of organic matter, was introduced into a flask with 20 g. of pure arsenic-free zinc and a quantity of pure concentrated hydrochloric acid sufficient to secure a brisk evolution of hydrogen. Diluted sulphuric acid (1 in 4 by volume) could also be used, but only after destruction of organic matter. Trouble was soon experienced with the zinc, some samples of which were found to be insensitive. They gave the brisk and regular evolution of hydrogen which is required for the Marsh-Berzelius method (p. 189) but failed to reduce all the arsenic to arsine, in spite of the high position occupied by zinc in Caspari's hydrogen overvoltage series. Chapman and Law (1906) concluded that this was due to the presence in the zinc of particles of more electro-positive metals with low overvoltage, which formed electric couples with the zinc and acted as a number of minute cathodes at which hydrogen was liberated at a tension too low to reduce either arsenic acid or arsenious acid. They found that if a salt of a metal such as cadmium, electro-positive to zinc and of high overvoltage, was added to the flask contents the sensitivity of the zinc was immediately restored, owing apparently to the deposition of cadmium upon the particles of foreign metals, thus preventing the liberation of hydrogen at a low tension. Tin, which also has a high overvoltage, can be used instead of cadmium, and 'stannated' hydrochloric acid containing stannous chloride is officially prescribed for use in the British Pharmacopœia method for arsenic determination. Stannous chloride has the additional advantage of reducing arsenic acid to arsenious acid if the temperature is high enough.

Evans (1920), in a study of the chemistry of the Marsh-Berzelius method, suggested that the insensitiveness of zinc may be due in part to the separation of elemental arsenic. Reduction of arsenious acid to elemental arsenic takes place more quickly than that of elemental arsenic to arsine. Sometimes elemental arsenic becomes detached from the zinc and collects as a film or scum on the surface of the liquid, where it is out of reach of further reduction. When a cadmium salt is added most of the metallic cadmium separates as a sponge and rises to the surface, where it comes into contact with the metallic arsenic and forms with it an electric couple in which arsenic, being the cathode, is rapidly reduced to arsine. If the zinc contains copper, copper arsenide may be formed as well as elemental arsenic. This can be reduced to arsine in the same way, provided that it comes into contact with cadmium.

Dyer (1920) and Taylor and Hamence (1942) refer to the use of a zinc-copper alloy containing 0·3 per cent of copper in place of pure zinc, and find that the addition of copper increases the reducing power of zinc and ensures complete reduction of arsenic acid to arsine. Normally copper would be expected to lower the overvoltage and therefore the reducing power. It is not clear to what the action of the copper is due. Possibly a copper-zinc alloy containing only 0·3 per cent of copper has a high overvoltage comparable with that of pure zinc, and the presence of copper may ensure a more regular and uniform reaction without loss of reducing power (cf. Mai and Hurt 1904, de Vamossy 1906). Dyer, when using the Marsh method, added cadmium sulphate to the flask containing the copper-zinc alloy. Taylor and Hamence, when using the Gutzeit method, dispense with a cadmium coating but invariably add stannous chloride to the generating flask.

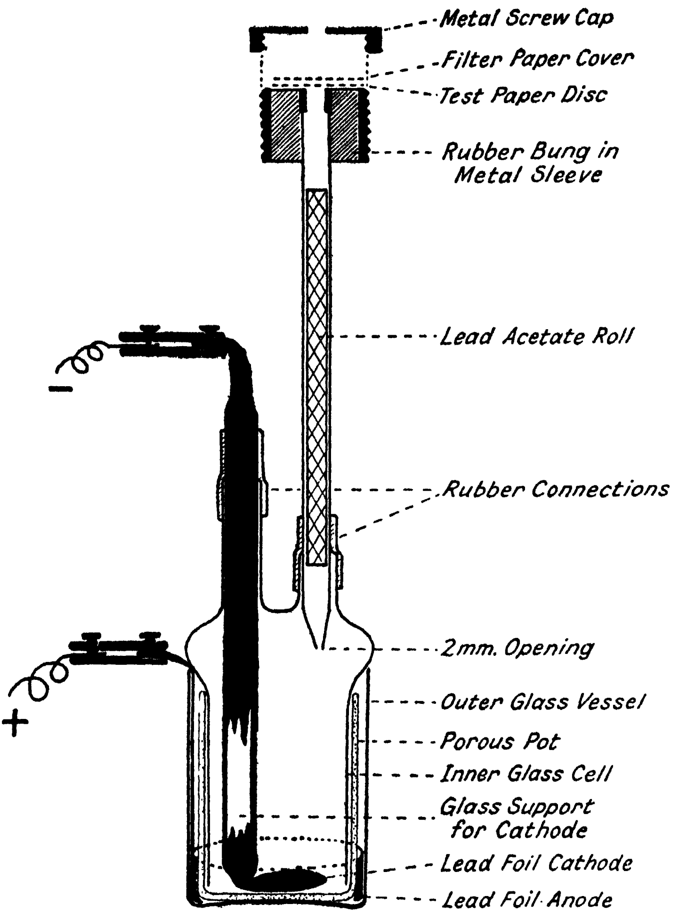
Temperature has a great effect in promoting reduction by nascent hydrogen, as was found by Kaufler in 1907 when using platinum electrodes for the reduction of organic compounds. Many of the contradictory statements by various authors concerning the conversion of arsenates to arsenites may be due to differences in the temperature of the liquid. Bodnár and others (1938) find that spongy tin and hydrochloric acid will reduce arsenic acid to arsenious acid at boiling-point but not at ordinary temperatures, and in several recent methods reduction is carried out with zinc and acid at or near the boiling-point of the liquid (Deemer and Schricker 1933, Allcroft and Green 1935, Cassil and Wichmann 1939). Some workers consider that reduction is facilitated by an increase in the effective surface of the zinc. Gangl and Sanchez (1934) use zinc dust instead of granulated zinc and heat the liquid. The use of a hot zinc and acid mixture with rapid evolution of hydrogen requires special methods of separating and determining the small quantities of arsenic carried forward by the hydrogen, such as passing the gas through a heated spiral capillary silica tube or through suitable absorbents followed by titration. Small amounts of copper salts are often used to promote the evolution of hydrogen, the possible adverse effect of copper on the reducing efficiency of zinc being offset by the addition of stannous chloride.

ELECTROLYTIC METHODS. In the reduction of arsenic compounds to arsine by electrolysis it is important to keep the arsenic close to the cathode of the cell and prevent it from becoming re-oxidised to arsenic acid by contact with the anode. Otherwise it may take a long time (3 hours instead of half an hour) for the arsenic to be reduced (Mai and Hurt 1905). It can be kept away from the anode, although not completely, by a parchment paper diaphragm or a porous earthenware pot separating the cathode and anode compartments. Even with this precaution a certain amount of arsenic will reach the anode either by diffusion or by anodic migration. Diffusion may be appreciable with a parchment diaphragm, especially if of large area ; with a porous pot it is small. Anodic migration is negligible with arsenious acid, but it may be considerable with arsenic acid owing to its higher degree of ionisation. It is therefore important that if arsenic acid is to be reduced the cathode shall be capable of doing this quickly before the loss due to anodic migration becomes appreciable.

In Thorpe's method, devised in the course of work for the Royal Commission in 1901–3, the electrodes are of sheet platinum. The current flowing through the cell is 5 amperes and the current density about 0·5 amperes per cm.^{2}. Platinum being a metal of low hydrogen overvoltage it is not surprising that this method fails to reduce arsenic acid to arsenious acid. This preliminary reduction must therefore be done beforehand with sulphur dioxide or other reducing agents. Platinum electrodes are apt to become insensitive after long use, probably owing to alteration in the state of the surface, but may be improved by passing a current through the cell in the reverse direction (Roche Lynch 1922). A further disadvantage of platinum is that it produces a good deal of hydrogen sulphide by reduction of the sulphuric acid of the electrolyte. Trotman (1904) suggested the addition of a zinc salt whereby the cathode becomes coated with zinc and is virtually a zinc electrode.

Several of the electrolytic methods now in use employ platinum anodes and lead or mercury cathodes, which have a high overvoltage and can reduce arsenic acid to arsenious acid. Lead cathodes have been found to be erratic in behaviour, owing possibly to a coating of sulphate or oxychloride or to an increase of effective surface so that hydrogen saturation is not so readily reached and overvoltage is lowered. The presence of substantial amounts of copper, iron and other metallic salts may cause a serious loss of sensitivity of lead cathodes (Callan 1924).

Many suggestions have been made for the treatment of lead electrodes to enable them to retain their initial sensitivity and reducing power, such as scouring and polishing, treatment with dilute nitric acid (Callan 1924), addition of a trace of cadmium salt to the electrolyte, and amalgamation of the lead, but none of these will enable sensitivity to be retained indefinitely. In the method described by me in 1923 the cathode is a strip of pure lead foil, the so-called ‘ assay ’ lead foil, terminating in a disc about 3 cm. in diameter, and the anode a similar strip of lead foil bent round the outside of the porous pot (cf. figure on p. 180). The cathode is renewed for each determination and is never in use long enough to become insensitive. Even so, however, it is advisable before using the electrodes to dip them momentarily into warm diluted nitric acid (1 of acid to 2 of water), which slightly etches the surface, and afterwards to wash them well with distilled water. If much iron is present in the solution under examination its effect can be neutralised by the addition of one or two grammes of dried milk, the phosphoprotein of which probably forms with iron a non-ionised ferric complex (p. 241). No doubt interference by iron might also be prevented by adding a little metaphosphoric acid. The internal resistance of the cell is about 3 ohms and the current used is 4 to 5 amperes. It has been found best to operate the cell without external cooling and to allow the temperature to rise to 85° or 90°. The current density at the cathode is relatively high, about 0·6 amperes per cm.^{2}. Lawson and Scott's failure to obtain reduction of arsenic acid to arsenious acid with a lead cathode may possibly have been due to the low current density which they used.



ELECTROLYTIC GUTZEIT APPARATUS
(Half Scale)

Mercury has the highest hydrogen overvoltage of all metals and has been successfully used as a cathode by Ramberg (1918) and by Aumonier (1927), who regard it as the most efficient metal for this purpose (cf. also Callan and Parry Jones 1930). In Aumonier's method the mercury cathode is contained in a porous pot the lower part of which is impregnated with wax. A platinum foil anode is used, with a current of 3 amperes and current density 0·22 amperes per cm.^{2}. The mercury is purified by distillation under reduced pressure. The arsenic is introduced into the cell in the form of arsenic acid. Excellent results are claimed for this method which has been in use for a number of years. Rogers and Heron (1946) describe an improved form of electrolytic cell with mercury cathode, so designed that hydrogen is evolved at a pressure sufficient to drive it through a solution for absorption of arsine (*Analyst*, **71**, 414).

Sand and Hackford (1904), on the other hand, failed to get any arsine at all either from arsenite or arsenate with a mercury cathode. They concluded that reduction to elemental arsenic took place and that this became amalgamated with mercury and escaped further reduction. Gangl and Sanchez (1934) found that in the zinc and acid method the addition of mercury salts completely stopped the formation of arsine. These contradictory results are difficult to explain. Ramberg found that with a mercury cathode the intermediate stage of reduction, arsenious acid to elemental arsenic, is much the quickest. Unless the arsenious acid is added very slowly it may be reduced so quickly to elemental arsenic that there is insufficient time for this to be converted into arsine and much of it is taken up by the mercury. There is not the same danger of this happening when arsenic is added in the form of arsenic acid, since the reduction of arsenic acid to arsenious acid is much slower. Sand and Hackford used a high current density, 0·6 ampere per cm.^{2}, and it is possible that under the conditions of their experiment elemental arsenic was produced at a speed at which it could not be converted into arsine before becoming amalgamated with mercury. Aumonier uses a lower current density, 0·22 amperes per cm.^{2}. Differences in current density and in speed of reduction are not, however, the explanation of the difference in results, since the mercury method has been found by me to work perfectly well both with arsenate and arsenite in amounts of the order of 0·005 mg., the cathode area being varied so as to produce current densities of 0·6 and 0·2 amperes per cm.^{2}, and without taking precautions to add the arsenic slowly. Possibly temperature may have a good deal to do with it. The complete reduction of arsenic acid to arsine is probably contingent upon the concentration of arsenic not exceeding a certain limit in relation to the active area of the mercury cathode and to the current density at a given temperature.

Zinc, tin, silver, iron and copper cathodes were tried by Ramberg, who found that they were all fairly efficient with large amounts of arsenic (2 to 5 mg.). For quantities of the order of 0·01 mg., copper was found by Sand and Hackford to be extremely inefficient. None of these metals has come into use for ordinary work, although a pure zinc cathode was used by Thomson (1904). Lockwood (1939) uses a cadmium cathode in conjunction with a continuous electrolytic method for control of raw materials used in confectionery manufacture. Amalgamated electrodes (silver, zinc, tin, cadmium) have also been used. They are sensitive when first amalgamated, but rapidly deteriorate and become insensitive in about two days. Torrance (1938) has found that with low voltage and weak currents, carefully controlled, metallic arsenic can be deposited quantitatively with copper without appreciable reduction to arsine, if hydrazine is added as a depolariser. Kaufler (1907) found that reduction at the cathode was facilitated by higher temperature—in fact he was inclined to regard the increased reducing power shown by electrodes with high hydrogen overvoltage as due mainly to local production of heat at the electrode surface.

Materials such as baking-powders and acid calcium phosphate sometimes fail to give satisfactory results in the electrolytic method with a lead cathode. This appears to be due simply to the immobilisation of sulphuric acid as calcium sulphate with the result that the electrolyte in the cell consists largely of phosphoric acid. It is important that the cell should contain sulphuric acid in the proportion of 1 volume of acid to 8 of water in addition to any phosphoric acid that may be present. If this strength of sulphuric acid is maintained, free phosphoric acid does not interfere with reduction.

A word of warning may be given on the use of electrolytic cells in which the cathode is suspended from a metal hook. The cathode chamber, soon after the electrolysis is started, becomes filled with an explosive mixture of air and hydrogen which may be ignited if sparking occurs at the point of suspension of the cathode. This may be prevented by inserting a drop of sulphuric acid at this point. With a lead-foil cathode for the same reason the strip of lead above the surface of the liquid must be stout enough to carry the current without fusing.

A disadvantage of electrolytic methods generally is that it is not always easy to obtain satisfactory porous pots.

DESTRUCTION OF ORGANIC MATTER. Foods in which arsenic is known to exist in inorganic form can often be tested without previous destruction of organic matter either by the zinc and acid method, or electrolytically using solid cathodes. If arsenic is present in organic combination organic matter must first be destroyed, and in general this course is preferable except in routine control work. With the mercury cathode previous destruction of organic matter is always necessary, otherwise the surface of the mercury cannot be kept clean. Oxidation is usually effected with sulphuric and nitric acids. The material should first be heated with concentrated nitric acid until practically dissolved. A small quantity (5 to 20 ml.) of sulphuric acid is then added, followed by successive small additions of nitric acid whenever the mixture shows signs of charring. Heating is continued until no further darkening takes place. Several workers have insisted on the necessity of keeping the mixture fully oxidised during the whole operation to avoid loss of arsenious oxide. Gautier's method, adopted by the Joint Committee in 1902, in which the material is charred with nitric and sulphuric acids and the char extracted with dilute hydrochloric acid, is alleged to give low results. Unless the charring is done below red heat sulphur dioxide may be formed and may reduce some of the arsenic to trioxide which is volatilised. Moreover, some arsenic may be retained by the carbon during extraction (Callan 1924). On the other hand, good results have been obtained by heating with sulphuric acid and acid potassium sulphate using copper sulphate as a catalyst, under conditions which are reducing rather than oxidising (Lawson and Scott 1925). It would seem that in presence of any considerable quantity of salt, arsenic might be lost by volatilisation as trichloride.

Allcroft and Green (1935) have examined critically the various methods of wet oxidation. Hydrochloric acid and potassium chlorate gave low results, being troublesome and liable to lead to loss of arsenic by volatilisation as chloride. Sulphuric acid alone also gave low results owing probably to volatilisation of arsenic trioxide. Nitric acid in conjunction with sulphuric acid was satisfactory provided that the precautions outlined above were observed. Allcroft and Green prefer a nitric and perchloric acid mixture which is quicker than the others and appears to involve the least loss of arsenic. Even so, the recovery of arsenic with quantities of the order of 0·01 mg. was only about 80 per cent and with 0·005 mg. not more than 60 per cent. It seems possible that these losses may have been incurred in the later stages of the determination and not in the wet combustion process.

Cassil (1940) refers to the fact that certain foods contain materials which resist acid digestion and states that organic matter may be completely destroyed by reheating the residue with perchloric acid. Fats especially are difficult to oxidise completely with sulphuric and nitric acids alone (p. 85).

Alternatively, organic matter can be destroyed by the so-called ‘ basic ’ method of ashing with an excess of pure calcium or magnesium oxide with or without magnesium nitrate, the ash being afterwards dissolved in dilute hydrochloric or sulphuric acid. Allcroft and Green found this method satisfactory provided that abundant excess of magnesium was added. Bamford (p. 213) uses 35 to 40 ml. of saturated magnesium nitrate solution for every 100 g. of material.

REDUCTION. The acid liquid remaining after wet oxidation with nitric and sulphuric acids may retain nitrogen acids tenaciously. If submitted to the Marsh-Berzelius test without further treatment these residual nitrogen acids may retard or even prevent the reduction of arsenic acid. Wilkie (1923) indeed maintained that the presence of nitrosulphonic acid even in minute amounts makes the reduction of arsenic acid to arsenious acid impossible. Dilution with water and reconcentration cannot be depended upon to remove nitrogen acids,[1] but they can be completely got rid of by adding 25 ml. of saturated ammonium oxalate solution and heating until the sulphuric acid fumes strongly (Ramberg 1918). This treatment does not reduce arsenic acid to arsenious acid. After removal of nitrogen acids in this way arsenic acid can be reduced to arsine, although more slowly than arsenious acid, either by zinc and acid or by electrolysis using lead or mercury cathodes.

In this connection it is of interest to note that the Joint Committee in 1902 stated emphatically that arsenic in both states of oxidation could be determined by the zinc and acid method, whereas now it seems to be held that previous reduction of arsenic acid is always necessary, whether for the zinc and acid or the electrolytic method (Davis and Maltby 1936). This apparent contradiction may be due to the fact that the Joint Committee used Gautier's method of oxidation in which the mixture is charred and all nitric acid driven off. In the wet oxidation method now generally used, nitrogen acids may be retained by the sulphuric acid. Failure to get satisfactory results has been attributed to the difficulty of reducing arsenic acid, but is probably due in part to the oxidising action of residual nitrogen acids.

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[1] G. Lunge (*Sulphuric Acid and Alkali*, 1903, vol. I, p. 224) says: 'Most of the nitric acid present along with nitrosulphuric acid in dilute acids (of sp. gr. 1·5 and under) remains behind in the liquid even after prolonged boiling. If, therefore, the nitrous vitriol of acid works, in consequence of a faulty process, contains nitric along with nitrous acid, it cannot possibly be completely denitrated by hot water or steam . . . ; the denitration can be effected only by reducing agents, such as sulphurous acid. . . .'

Foods contaminated with arsenical war gases may present special difficulties (Williams 1941, Stainsby and Taylor 1941).

As has already been pointed out, a possible factor in preventing reduction of arsenic acid is the presence of iron or other metals of low supertension, which may form a couple with the zinc or be deposited on the cathode and thus affect the reducing power. Traces of iron are very likely responsible for the insensitiveness of some samples of zinc. If arsenic acid is previously reduced to arsenious acid, conversion to arsine can be effected at a lower overvoltage and the presence of iron in moderate amount does not have the same inhibiting effect. Temperature also may be one of the controlling factors. If the temperature rises to 85° or 90°, reduction of arsenic acid may be effected far more readily than if the vessel is cooled externally with water (cf. p. 180).

Reduction may be carried out with sulphur dioxide, stannous chloride, potassium iodide or bromide, hydrazine sulphate, etc. (S.P.A. Sub-committee 1930). Hydrazine sulphate has the advantage of reducing nitrogen acids to nitrogen. Davis and Maltby (1936) find that sulphur dioxide is the simplest to use and the most certain to ensure reduction of the whole of the arsenate. They emphasise the necessity of using a sufficient excess. Reduction is carried out on a steam bath, and residual sulphur dioxide must then be completely removed by boiling for two or three minutes, otherwise an excessive amount of hydrogen sulphide will be produced during the evolution of arsine. This hydrogen sulphide might have the effect of immobilising arsenic as an insoluble sulphide and thus removing it from the reducing action of hydrogen. It should be borne in mind that some reducing agents, e.g. stannous chloride, may under certain conditions reduce arsenic acid to elemental arsenic (p. 176) and thus lead to low results.

When potassium iodide or bromide are used the conditions must be such that the iodine or bromine produced does not re-oxidise arsenious acid. Wilkie (1923) removed iodine with either terpin hydrate or ‘ oxidised glycerol ’.

DISTILLATION. Distillation of arsenic as trichloride is recommended by several workers as a means of separating it from all substances likely to interfere with subsequent reduction to arsine. Originally it was used only for materials such as fuel containing a great deal of ash which could not easily be dealt with in the ordinary way. Aumonier (1927) emphasises the necessity of distillation in all cases to avoid 'poisoning', both in the Marsh-Berzelius and the electrolytic methods. Ramberg and Bang in their work for the Swedish Arsenic Commission (Cox 1925) used a distillation method after reduction with ferrous sulphate and potassium bromide, but they dealt with relatively large amounts of arsenic and determined it in the distillate by titration. In the Official Methods (1945) of the Association of Official Agricultural Chemists, distillation is recommended whenever it is desirable to concentrate arsenic, when interfering substances such as pyridine residues from insecticides are present in the digest or when the sample contains excessive amounts of salts or sulphuric acid from wet oxidation: with food products the distillation method is reserved for amounts of arsenic of at least 0·3 mg. Distillation is also used, in conjunction with hydrazine and potassium bromide reduction, to separate arsenic from residues after wet oxidation of food colours which may contain considerable amounts of other metals (S.P.A. Analytical Methods Committee 1930).

Distillation must be carried out in such a way that hydrochloric acid does not come into contact with cork or rubber, which may contain arsenic. The arsenious acid in the distillate may be titrated direct or be re-oxidised with nitric acid or bromine to prevent loss of arsenic during concentration and the excess of nitric acid or bromine completely removed on the steam bath. The residue of arsenic acid is taken up with dilute hydrochloric or sulphuric acid and introduced into the generating flask or electrolytic cell. If reduction to arsenious acid is considered necessary at this point it can be effected with stannous chloride.

When ferrous sulphate is used as reducing agent before distillation of arsenic as trichloride, sulphur dioxide may be produced from the ferrous sulphate if the distillation is taken too far. This is of no consequence when the distillate is to be oxidised and evaporated, but may interfere with direct titration of arsenic in the distillate (Schaaf and Maurer 1943). On the other hand, if nitric acid or nitrates are present, nitrosyl chloride (NOCl) may distil over with the arsenic trichloride and re-oxidise some of it in the distillate (Graham and Smith 1922).

Magnuson and Watson (1944) find that arsenic can be obtained in the quinquevalent form by distillation if no reducing agent other than potassium bromide is present. Organic matter is destroyed by oxidation with sulphuric and nitric acids, and residual nitric acid is completely removed. The liquid is distilled in a special distilling flask with sulphuric acid and potassium bromide and the vapour passed through a hot-water trap which retains the arsenic in a small volume of distillate. The arsenic is found to be in the quinquevalent condition. Magnuson and Watson suggest that it may be volatilised as pentabromide which gives arsenic acid on hydrolysis. It is determined colorimetrically by the molybdenum blue method (cf. Chaney and Magnuson, p. 198). The authors claim that by this method 1 μg. of arsenic can be determined to within ± 10 per cent, and 5 μg. to within ± 5 per cent, and that the results are trustworthy and consistent.

Separation of Arsenic Trioxide with Diethylammonium Diethyl-dithiocarbamate. This reagent has lately been introduced by Strafford and others (1945). It is prepared by the interaction of diethylamine and carbon disulphide in chloroform and has the formula $CS\begin{smallmatrix}\diagup N(C_2H_5)_2 \\ \diagdown S.NH_2(C_2H_5)_2\end{smallmatrix}$ (cf. the sodium salt, p. 49). When a chloroform solution of this compound is shaken with a solution of arsenic trioxide in 2N hydrochloric acid the arsenic combines with the thiocarbamate and passes into the chloroform. Arsenic pentoxide does not combine with the thiocarbamate so that this process gives a sharp separation of trioxide from pentoxide. Copper, mercury and bismuth also react with this compound in acid solution under similar conditions: lead does not react in 2N HCl (cf. pp. 88, 129).

Other thio-compounds which have been used in a similar way for the separation of arsenite from arsenate are sodium xanthate $CS(OC_2H_5)SNa$ (Crawford and Storey 1944) and ethane-1:2-dithiol (Crawford and Levvy 1946). The application of these thio-compounds in analysis is of interest in connection with the newer views on the toxicology of arsenic and heavy metals (pp. 11, 163).

PRODUCTION OF ARSENIC MIRRORS FROM ARSINE. In the Marsh-Berzelius method as developed by the Joint Committee in 1902 the hydrogen is passed first through a tube containing granulated calcium chloride and a roll of lead acetate paper to remove moisture and hydrogen sulphide. The calcium chloride must be pure; some samples have been found to retain arsine. The use of paper for holding the lead acetate has been criticised on the ground that acid spray may be carried on to it by the gas stream and that the acid partly hydrolyses cellulose giving a product which absorbs arsine. The extent to which this may happen will depend largely upon the design of the apparatus. It can be prevented by substituting glass wool or coarse sand for the paper (Ward 1926). It has been stated by Reckleben that lead acetate may combine with and retain arsine, and for this reason Aumonier (1927) omits lead acetate altogether when working with a mercury cathode which gives very little hydrogen sulphide. Reckleben, however, worked with hydrogen containing 16 per cent of arsine; with very small quantities of arsine in hydrogen there is not the same danger of its retention by lead acetate. Gangl and Sanchez (1934) found, under the conditions adopted by them, a loss of 3 to 4 per cent of arsenic from absorption by lead acetate.

The calcium chloride-lead acetate tube is connected to a hard glass tube drawn out to a capillary of 1 to 2 mm. internal diameter. This tube is heated by a small Bunsen flame over a short distance just before it narrows to form the capillary. Arsine is decomposed at temperatures of 250° to 300°, and a brown deposit or mirror of metallic arsenic is deposited in the capillary tube about a centimetre from the edge of the flame. It is important that all air should be expelled from the flask by hydrogen before introduction of the solution containing arsenic, otherwise the mirror will be discoloured and uneven. The hard glass tube is apt to sag towards the end of the operation. Its collapse may be prevented by wrapping a small piece of platinum gauze round the part exposed to the flame. Aumonier (1927) uses, instead of a gas flame, a small electric heater in which the temperature is controlled at 800° and a steadier and more uniform heat is obtained. The amount of arsenic is estimated by comparison with standards obtained under similar conditions. According to the Joint Committee it is possible, with a total amount of arsenic in the region of 0·01 mg., to detect differences of 0·002 mg.

The formation of an arsenic mirror is favoured by quick cooling of the gas. With 0·001 mg. it often happens that no recognisable deposit is obtained, whereas if the capillary is water-cooled by wrapping a strip of wet filter-paper round it, thus making the heat gradient sharper, this quantity of arsenic gives a well-defined mirror. Again, if the capillary tube is covered for half an inch with asbestos paper so as to prolong the heat gradient, the mirror obtained with 0·01 mg. is far less well defined and more diffuse than if the heat gradient is steep.

The mirror does not represent the whole of the arsenic present, the loss being relatively greater with the smaller quantities, but this is of no consequence if the mirrors are compared with standards obtained under exactly similar conditions. Gangl and Sanchez (1934) discuss the conditions necessary for the complete decomposition of arsine and deposition of arsenic. They use a spiral silica capillary tube which is heated throughout the whole of its length. The arsenic deposit is subsequently dissolved in iodine monochloride and titrated (p. 196; cf. also Szép and Cieleszky 1939).

Certain other elements, if present, may also give deposits in the tube. Antimony hydride is more easily decomposed by heat than arsine, and the mirror is partly deposited in front of the flame. It is blacker than that produced by arsenic. Sanger and Gibson (1907) have made a study of the determination of antimony by the Marsh method, for details of which their paper should be consulted ; also a paper by Grant (1928). Selenium and tellurium also give mirrors at a lower heat than arsenic, that of selenium being reddish in colour (p. 235). Both selenium and tellurium hydrides resemble hydrogen sulphide in being retained by lead acetate paper. Tin will also give a mirror in the electrolytic method with lead cathode when organic matter is present, as for instance when amyl alcohol is used in the cell to prevent frothing. These tin mirrors are sometimes obtained when testing Demerara sugar containing about 1 gr. of tin per lb. without destruction of organic matter, and are due to the formation and decomposition of tin hydride, SnH_4 (Paneth and Rabinowitsch 1924). The decomposition takes place at a temperature lower than that of arsine, and the mirror may form on both sides of the flame. Germanium also gives a mirror under certain conditions.

A disadvantage of the Marsh-Berzelius method as applied to the very small amounts of arsenic found in foods is the difficulty of getting suitable capillary glass tubes. When a stock of tubes is exhausted it is sometimes impossible to secure the same type and thickness of tube again. In making the capillaries much practice is required to obtain uniformity of dimensions.

GUTZEIT METHOD. This method depends upon the production of a yellow to orange-brown colour when arsine comes into contact with mercuric chloride or bromide. The reaction was first noted by Mayençon and Bergeret in 1874, and was developed on a quantitative basis by Gutzeit a few years later. The strength of acid used in the generating flask is lower than in the Marsh-Berzelius method, since it is of advantage to have a slower evolution of arsine. The hydrogen gas containing arsine is freed from hydrogen sulphide with lead acetate paper and is then passed through, or over, paper impregnated with mercuric chloride or bromide.

The colour and intensity of the stains produced are compared with standards. Mercuric bromide is the more sensitive of the two. There is some difference of opinion whether it is better to pass the gas along a tube containing a strip of mercuric bromide paper (Sanger and Black 1907, Hollins 1917, Crossley 1936) or to make the gas pass through the paper. In the one method the stain is formed on the surface of the paper and the amount of arsenic estimated from the length and intensity of the stain. In the other, a more porous paper must be used to admit of free passage of gas, and some of the colour may be formed in the interior of the paper and not be evident. In both there is a possibility that the mercuric bromide on the paper may not retain all the arsenic, but if the stains are compared with standards obtained under similar conditions, this source of error is eliminated. In general, the method of passing the gas through a vertical glass tube closed at the top with a disc of filter-paper impregnated with mercuric bromide is the one most favoured. A number of devices have been suggested for holding the paper in position in such a way that all the gas must pass through it without escaping round the edge, and thus form a sharply defined circular stain (Jones 1919, Stubbs 1927, White 1927, Scott Dodd 1928, Richmond 1928, Manley 1929, Linsey 1930). A satisfactory device is the one described in a report of a sub-committee of the Institute of Brewing Standing Committee for Analysis (1938). This report, which is reproduced in the *Analyst*, gives an admirable account of the Gutzeit test with full working details. Since the mercuric bromide stains are not permanent the sub-committee adopt Henley's method (1928) of comparing them with a series of water-colour stains made from mixtures of chrome yellow, chrome orange and burnt umber in varying proportions. The use of permanent water-colour standards is a valuable improvement and makes the Gutzeit method preferable in many respects to the Marsh-Berzelius. The standards are best prepared by making a number of different tints with varying mixtures of the colours. From these a set can be chosen to match the stains obtained in tests made with known amounts of arsenic. The set is mounted on cardboard and kept for reference. For general work a series of standards ranging from 0·001 to 0·01 mg. is preferable to the series given in the Institute of Brewing report.

In routine examination of brewing materials (sugar, malt, hops, beer and finings) by the Gutzeit method, the sample is usually tested direct without previous destruction of organic matter. In general, however, it is preferable to destroy organic matter by wet oxidation or ashing, and in this form the method is particularly well suited for use with an electrolytic cell, since the rate of gas evolution is so completely under control. The figure on page 180 shows an electrolytic cell with lead electrodes as originally devised for the Marsh-Berzelius method (Monier-Williams 1923), but simplified and adapted for use with mercuric bromide paper. It is not necessary, when using mercuric bromide paper, to displace air in the cell before adding the liquid to be tested, so that the tap funnel usually fitted to the electrolytic cell is omitted. The vertical glass tube containing the roll of lead acetate paper is drawn out to a small opening at its lower end to minimise the risk of acid spray reaching the paper. The preparation and fitting of the mercuric bromide paper are as given in the Institute of Brewing report. 20 ml. of the solution to be tested, containing one part of sulphuric acid by volume to eight parts of water, and if necessary 1 ml. of amyl alcohol to prevent frothing, are placed in the porous pot and 10 ml. of diluted sulphuric acid (1 vol. of acid to 8 of water) in the anode compartment. This latter quantity will, of course, depend on the closeness of fit of the porous pot in the beaker. The level of acid in the porous pot should be somewhat higher than that in the anode compartment. The apparatus is then assembled and a current of 4 amperes passed through the cell for 30 minutes, by which time all the arsenic present should have been evolved as arsine and brought into contact with the mercuric bromide paper. A similar form of apparatus has been described by Evers (1926).

Antimony gives a stain quite different from that given by arsenic, and the test as applied to antimony is less delicate.

No stain is obtained in the electrolytic method with lead cathode unless at least 0·04 mg. of antimony oxide is present. This produces a brownish stain. With 0·06 mg. the stain is greyish-black and of intensity almost equal to that given by 0·10 mg. Thus with antimony there is neither the sensitivity nor the gradation shown by arsenic.

If both antimony and arsenic are present in foods, which might occur, for instance, in a food prepared or stored in cheap enamelled vessels, they may be separated by magnesium phosphate precipitation. The acid liquid after wet oxidation with nitric and sulphuric acids is diluted and neutralised with ammonia and about 20 ml. of 10 per cent sodium phosphate solution added. Magnesia mixture is then added slowly with shaking and the liquid allowed to stand. Arsenic is completely precipitated as magnesium ammonium arsenate together with the phosphate, leaving all the antimony in solution. The precipitate is filtered off, dissolved in dilute hydrochloric or sulphuric acid and submitted to the Gutzeit test after reduction. The remarks on page 182 on the presence of phosphoric acid should be noted. This method is often used irrespective of antimony in the sample, for instance by the Association of Official Agricultural Chemists in the determination of arsenic in food colours. Reiss (1919) precipitates arsenic as aluminium arsenate from a diluted solution with aluminium sulphate and ammonia.

Antimony can also be separated from arsenic by using tin instead of zinc in the Marsh-Berzelius or Gutzeit methods. Antimony is not reduced to hydride by tin and hydrochloric acid and remains in the flask (Tananaeff and Ponomarjeff 1935, Bodnár and others 1939 ; cf. also p. 217).

TITRATION METHODS. The arsenic mirror obtained in the Marsh-Berzelius method can be dissolved, although with some difficulty, in 2 ml. of 0·0005N iodine solution (Ramberg 1919, Lendrich and Mayer 1926). The reaction is

$$\mathrm{As} + 5\mathrm{I} + 4\mathrm{H_2O} = \mathrm{H_3AsO_4} + 5\mathrm{HI}.$$

Sodium bicarbonate and starch are added and the excess of iodine titrated with standard arsenite solution. Alternatively 2 ml. of 0·0005N sodium arsenite solution may be added and the excess of arsenite titrated with standard iodine solution. A capillary burette is used similar to that described on page 329. 1 ml. of 0·0005N iodine solution is equivalent to 0·0075 mg. of metallic arsenic. Unless the hydriodic acid formed is neutralised the oxidation of arsenic, as is well known, is not complete, the reaction between arsenious acid and iodine being reversible. In presence of bicarbonate oxidation to arsenic acid is quantitative. Billeter and Marfurt (1923) dissolve the arsenic mirror in iodine solution with bicarbonate and titrate the excess of iodine with thiosulphate. This procedure was criticised by Ramberg (1918) on the ground that it is wrong in principle to titrate iodine with thiosulphate in bicarbonate solution and that the amount of arsenic thus found is too high.

For very small amounts of arsenic the titration method is probably not more accurate than visual comparison of the mirrors. In titrating with these extremely weak solutions the dilution error is very great, and it is essential that the blank should be titrated under closely similar conditions of dilution and volume. Apart from the errors inherent in the titration of such small amounts, it is possible that the arsenic mirror is not of uniform composition but contains some suboxide As_2O and hydride AsH, which reduce different amounts of iodine. If they were present in equivalent proportions the net result would be the same as if the mirror consisted only of elemental arsenic, but if the relative amounts of As_2O and AsH vary the iodine reduced will not be strictly proportional to the arsenic present. Again with very small amounts of arsenic, of the order of 0·01 mg., deposition in the capillary tube is probably not complete (p. 190) and the titration method is therefore suitable only for relatively large amounts.

Ramberg (1918) absorbs arsine directly in standard iodine solution. Bicarbonate is then added and the excess of iodine titrated with standard arsenite solution. Iodine solution may not be fully effective in absorbing and oxidising arsine unless the solution is buffered with bicarbonate to neutralise hydrogen iodide as it is produced (Milton and Duffield 1942). The reaction between arsine and iodine may be represented as follows :

$$AsH_3 + 8I + 4H_2O = H_3AsO_4 + 8HI.$$

In all these iodimetric titrations it should be borne in mind that elemental arsenic, arsine and arsenious acid reduce different amounts of iodine.

When elemental arsenic or arsine act upon iodine they reduce it to hydriodic acid ; when they act upon iodine in a still higher state of oxidation, as iodine chloride, ICl, they reduce this first to iodine. This iodine can then be determined by Lang's method, in which it is titrated with an oxidising agent, such as potassium iodate, in presence of a cyanide. The iodine is oxidised to iodine cyanide ICN. The reactions are

$$As + 5ICl + 4H_2O = H_3AsO_4 + 5I + 5HCl.$$
$$HIO_3 + 4I + 5HCN = 5ICN + 3H_2O.$$

The titration is best carried out with 0·001M potassium iodate solution, and instead of starch as indicator two drops of carbon tetrachloride are added, the end-point being marked by the decolorisation of the carbon tetrachloride. 1 ml. of 0·001M iodate is equivalent to 0·060 mg. of metallic arsenic. One of the advantages of this method is that the arsenic mirror dissolves much more readily in iodine chloride solution than in iodine-potassium iodide solution. The titration may also be carried out with potassium permanganate instead of iodate. Alternatively, enough of the oxidising agent can be added to convert all the iodine into ICN, the excess of oxidising agent removed and the ICN determined with thiosulphate (Gangl and Sanchez 1934, Kubina 1929, Lang 1922, 1925).

Allcroft and Green (1935) find that traces of arsenic are absorbed from large volumes of hydrogen by 0·01N silver nitrate solution according to the equation

$$2AsH_3 + 12AgNO_3 + 3H_2O = 12Ag + 12HNO_3 + As_2O_3.$$

The absorption is extraordinarily efficient ; an extremely rapid current of hydrogen from zinc and hot dilute sulphuric acid is passed through the absorbent, and no arsenic is lost as long as the liquid is not blown out of the tubes. After addition of a small quantity of bicarbonate and sufficient potassium iodide to dissolve the silver iodide first formed, the arsenious acid is titrated direct with very dilute standard iodine solution. With amounts of arsenic down to 0·005 mg. Allcroft and Green find the results better than can be obtained by the Gutzeit method. They tested the method, in conjunction with nitric-perchloric acid oxidation, on milk, flour and beer to which about 1·4 p.p.m. ($\frac{1}{100}$ gr. per lb.) of arsenious oxide had been added, 10 g. of the sample being used in each determination. The recovery of arsenic was from 82 to 93 per cent. There is, however, no note of the amount of arsenic given by blank tests on these materials. A modification of Allcroft and Green's method is described by Levvy (1943), who also claims that it gives more accurate results than the Gutzeit method.

In Cassil and Wichmann's method (1939) arsine is absorbed in mercuric chloride solution, with production of arsenious acid and calomel. The mixture is titrated with standard iodine solution, the amount of iodine used representing the sum of the oxidation stages from arsine to arsenic acid, i.e. 1 atom of arsenic to 8 of iodine.

Several titration methods are combined with the preliminary distillation of arsenic as trichloride. In Bang's method, referred to by Cox (1925), the trichloride is distilled with hydrochloric acid into sodium hydroxide solution containing phenol-phthalein until this becomes acid. The distillate is then treated with excess of sodium bicarbonate and titrated direct with 0·0005N iodine solution. Ramberg distils arsenic trichloride into water and titrates the resulting arsenious acid with standard potassium bromate solution using methyl orange as indicator (Cox 1925). The end-point is reached when the red colour of the methyl orange is discharged by the potassium bromate. These methods are hardly applicable to the small amounts of arsenic usually found in foods. The bromate method is suggested by the Association of Official Agricultural Chemists for amounts of arsenic in excess of 0·3 mg. Von Fellenberg (1930) precipitates arsenic as sulphide after distillation as trichloride and titrates the sulphide with potassium permanganate in alkaline solution. The results with small quantities of arsenic are rather high.

COLORIMETRIC METHODS. The best known colorimetric method for arsenic is that developed by Zinzadzé (1930, 1932, 1935) from the original test given by Osmond in 1887 and later by Denigès (1920). When a solution of molybdic acid is reduced with stannous chloride a blue solution of the compound Mo_2O_5, $4MoO_3$ is formed. This compound is unstable, especially in strongly acid solution, and the blue colour disappears on dilution. In presence of phosphoric, arsenic, silicic or germanic acids blue compounds are formed, e.g. $(Mo_2O_5, 4MoO_3)_2H_3AsO_4$, which do not lose their colour on dilution, the intensity of the colour being proportional to the amount of these acids present. The method is a delicate one, and 0·005 mg. of arsenic can be detected in 50 ml. of solution taken for colorimetric comparison. The reagent is a sulphuric acid solution of molybdic acid in which the amount of sulphuric acid is so adjusted that the solution on reduction with stannous chloride will be about normal strength. This is found to give the maximum development of colour without undue interference with reduction. At higher acidities, above 1·5N, reduction of arsenomolybdic acid by stannous chloride is inhibited. The concentration of molybdic acid and the amount of stannous chloride used must be the same in the test and comparison solutions. When the optical density of the blue solution is to be determined in a photometer the quantities of all the reagents must be carefully standardised (Milton and Duffield 1942). Chaney and Magnuson (1940) use hydrazine sulphate instead of stannous chloride as the reducing agent. Probably 1:2:4-aminonaphthol sulphonic acid, which is used as a reducing agent in phosphate and silicate determinations by the molybdenum method (pp. 131, 400) could be used with advantage for arsenate.

When determining arsenic in foods it is of course necessary first to separate it from phosphoric acid either by distillation as chloride or by evolution as arsine from zinc and acid or electrolytically, and absorption of the arsine in a mixture of nitric acid and bromine, iodine and sodium bicarbonate (Milton and Duffield 1942) or other oxidising agent. In Chaney and Magnuson's distillation method (1940) arsenic trichloride in the distillate is oxidised to arsenic acid with potassium iodate (see also Magnuson and Watson's distillation with potassium bromide, p. 188).

The blue colour produced by stannous chloride reduction of arsenomolybdic acid is not permanent. If molybdic acid is first reduced to molybdenum blue, Mo_2O_5, $4MoO_3$, by heating it with metallic molybdenum in concentrated sulphuric acid, and this reduced product afterwards combined with arsenic acid, the resulting blue compound is much more stable, although the method is perhaps slightly less delicate for arsenic. A molybdenum-blue reagent of this type can be prepared as follows (Zinzadzé). To 1,010 ml. of 25N sulphuric acid (sp. gr. 1·66 ; 74 per cent by weight) are added 40·1 g. of pure molybdic acid MoO_3. The mixture is boiled gently with occasional shaking until solution is complete, avoiding the evolution of white fumes. It is then cooled, diluted with distilled water, cooled again and made up to 1 litre. The solution is blue (solution I). 500 ml. of solution I are now reduced by adding 1·78 g. of metallic molybdenum powder and boiling the liquid gently for 15 minutes with occasional shaking. It is cooled, decanted from any residue and made up to 500 ml. This solution is dark green (solution II). 5 ml. of solution II are diluted to about 50 ml. and titrated with 0·1N permanganate solution. As the original solution is viscous it is necessary to wash it out of the pipette with water. On dilution with water it becomes orange in colour, and on addition of permanganate the colour gradually fades until the solution becomes colourless. The addition of permanganate is then continued until the end-point is reached. The molybdenum-blue reagent is finally prepared by mixing solution I with solution II in such proportions that 5 ml. of the mixture reduces 5 ml. of 0·1N permanganate. It is intensely blue and will keep for a long time provided that it is made up from pure materials and is kept free from dust in a glass-stoppered Pyrex glass bottle.

Alternative methods for preparing the solution have been given at different times both by Zinzadzé and other workers, but the above procedure gives a satisfactory reagent which keeps well. Schricker and Dawson (1939) have modified the method in several respects for the direct determination of phosphoric and arsenic acids in soil extracts. The composition of the molybdenum reagent in these various modifications has been arrived at more or less empirically with the object of securing the best conditions for the reaction with minimum interference from silicic acid and salts, and the amounts of molybdenum and sulphuric acid recommended vary to some extent with different workers. It seems to be important that the reduction of the molybdic acid should be carried to the right point for maximum blue production as determined by permanganate titration and that the acid concentration should be high enough to ensure the stability of the reagent and also the stability of the arsenic acid compound of molybdenum blue after dilution for colorimetry.

To determine arsenic in amounts from 0·005 to 0·3 mg. the required amount of the solution containing arsenic acid is introduced into a 50-ml. flask having a mark at 40 ml. A small quantity of α-dinitrophenol solution is added as an indicator, and the solution is neutralised, if acid, with a 2 per cent solution of sodium bicarbonate or, if alkaline, with normal sulphuric acid to a faint yellow colour. Some of the molybdenum blue reagent, prepared as above described, is then diluted tenfold and 5 ml. of this diluted reagent added to the solution in the flask. The reagent as diluted tenfold is orange, but on further dilution is colourless. The flask contents are made up to 40 ml., heated on a steam bath for 30 minutes, cooled and made up to 50 ml. The colour is then compared with a series of colours given by a standard arsenic acid solution. This standard solution is prepared by dissolving 0·02 g. of arsenic oxide As_2O_5 in about 5 ml. of 2 per cent sodium bicarbonate solution and 100 ml. of water:

6 ml. of normal sulphuric acid are added, followed by three drops of 0·1N permanganate, and the whole made up to 1 litre. One millilitre of this solution contains 0·02 mg. of arsenic oxide.

Fluorides interfere with the molybdenum blue reaction, but their effect may be minimised by the addition of a small quantity of boric acid (Kurtz 1942).

The application of the phosphomolybdic reaction to the determination of copper, zinc and tin is referred to on pages 46, 131 and 158.

Arsenic can be reduced to the metallic state by heating with certain reducing agents and the brown coloration or turbidity compared with standards. This method, with hypophosphorous acid as the reducing agent, has been used in France for determining arsenic in wines (Fleury 1923) and in glucose (Kling 1917). It was introduced originally by Thiele in 1890 and elaborated by Bougault in 1909 for arsenic in its organic compounds. It has been applied by Evans (1929) to the determination of arsenic in metals. Nitric and nitrous acids interfere with the reduction. Selenium and tellurium compounds are reduced more readily than those of arsenic by hypophosphorous acid (Challis 1941). Kling (1917) precipitates arsenic first as magnesium ammonium arsenate in presence of excess of phosphate and heats the precipitate with hypophosphorous acid. The limit of sensitivity of the method is given as 0·03 mg. of arsenious oxide in 1 ml. of solution. Harispe (1939) stabilises the colloidal arsenic precipitate with silicic acid, and Thuret (1939) uses artificial standards prepared by heating powdered colophony with a solution of borax. In Bettendorf's test a similar precipitation of elemental arsenic is effected by reducing with stannous chloride. This reaction has been used by Atkinson (1934) for determining arsenic in tartar emetic.

Kamerman (1939) passes the gas evolved from the Marsh apparatus through a small volume of N/100 silver nitrate solution containing gum arabic and compares the colour of the reduced silver sol with standards.

ARSENIC AND MOULDS. When certain moulds, especially the species *Scopulariopsis brevicaulis*, are allowed to grow on media containing arsenic a garlic-like smell may be produced due to the formation of volatile organic arsenic compounds. The gas evolved has been termed 'Gosio' gas from the name of the investigator who studied it in 1893. Gosio believed that the gas was diethylarsine $(C_2H_5)_2AsH$. Challenger (1935) considered it to be trimethylarsine. According to the Swedish Commission on arsenical poisoning (Petrén 1923) the gas is not of constant composition and is probably a mixture of several volatile compounds of arsenic. The smell of the gas has been stated to be a more delicate test for the presence of arsenic than the Marsh-Berzelius or Gutzeit tests (Remy and Richter 1929, Smith and Cameron 1933) but Lerrigo (1932) was unable to detect any volatile arsenic compounds whatever when moulds were grown by him on arsenical foods. It would seem that particular conditions may be necessary for the production of 'Gosio' gas and that unless the right mould species is available the test is somewhat uncertain. The study of this gas was the starting-point of a series of important researches by Challenger on biological methylation.

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6

[ANTIMONY]

ANTIMONY may gain access to food from (1) enamelled vessels, (2) solders and tin-foil containing antimony, (3) rubber rings and rubber tubing, (4) antimony compounds in insecticide sprays. Of these, the first named is the most important. On three occasions in this country, between the years 1928 and 1932, soluble antimony compounds derived from hollow-ware enamel have been responsible for poisoning on a considerable scale involving about 170 persons, fortunately without fatal results. Details of these outbreaks are given in a report to the Ministry of Health (Monier-Williams 1934).

TOXICITY OF ANTIMONY COMPOUNDS. The action of antimony compounds when ingested depends on the one hand upon their solubility and on the other hand upon their state of oxidation, that is, whether the antimony is in the tervalent or quinquevalent state. Tervalent antimony readily forms soluble compounds such as tartar emetic (potassium antimonyl tartrate). This compound in doses above 0·06 g. quickly causes vomiting, but if from any cause it is retained in the body it may give rise to severe poisoning. In sub-emetic doses of 0·01 to 0·06 g. it depresses the heart and in still smaller doses of 0·003 to 0·008 g. it increases perspiration. According to Calvery (1942), fatal results have followed ingestion of 0·15 g. Tartar emetic, if administered regularly to dogs in doses insufficient to cause vomiting, eventually kills the dogs (Cloetta 1911), and similar results have been obtained with cats. On the other hand, Oliver (1933), in an investigation into the health of workers exposed to antimony trioxide dust, found that these men suffered no ill effects although working without respirators. They were excreting large amounts of antimony in their faeces but none in their urine. He concluded that antimony trioxide is not absorbed from the alimentary tract owing to its insolubility. Presumably the dust was retained by the mucous membrane of the nose and throat in these workers, since according to Dernehl and others (1945) antimony trioxide is much more toxic when inhaled than when ingested. Flury (1927) found that solid antimony trioxide, pentoxide and sodium metantimonate in doses of 2 to 3 g. were innocuous to rats, although toxic effects were obtained with dogs and cats after repeated doses of 0·5 g. daily for a long period. Tartar emetic was far more poisonous, vomiting in dogs being caused by as little as 4 mg. if given in concentrated form.

Scheller (1926) prepared a 0·8 per cent solution of sodium metantimonate (quinquevalent antimony) in 2 per cent tartaric acid solution and gave 240 ml., corresponding to 1·2 g. of antimony, to a dog. The dog suffered no ill effects, although it excreted 82 mg. of antimony in its urine in 24 hours, indicating the absorption of about one-fifteenth of the amount ingested. Cloetta (1911) found that potassium pyroantimonate, which is soluble to some extent in water, was non-poisonous to dogs in doses of about 0·5 g. Brunner (1912) concluded that all compounds of tervalent antimony are poisonous and all those of quinquevalent antimony relatively harmless. Hassan (1938), in researches on the action of antimony compounds used in the treatment of kala-azar, found that quinquevalent antimony has a high rate of excretion in urine, while tervalent antimony is not so readily eliminated. In medication with tervalent antimony compounds much of the antimony is retained in the liver, with serious results in a few cases. It is stated that the accumulation of 200 mg. of antimony in the adult human liver is liable to cause death (Bamford 1934).

It may be concluded, therefore, that while the absorption of antimony compounds depends largely upon their solubility, the toxicity of antimony when absorbed depends upon its state of oxidation. The effect upon human beings of continued minute doses of antimony is not known, but it would seem that it must be classed among the more toxic elements and that its presence in solid food in amounts over, say, 5 p.p.m., and in liquids over 1 p.p.m., is very undesirable. The colouring of food with compounds of antimony is specifically prohibited under the Preservatives Regulations 1925–7.

ANTIMONY IN HOLLOW-WARE ENAMEL. Hollow-ware enamel is made up from some or all of the following ingredients: borax, felspar, quartz, fluorspar, cryolite, lime, magnesia, sodium carbonate, sodium nitrate and occasionally other materials. These are fused or fritted together in a furnace at 1,200° and the frit ground to powder. The powder is then mixed with water and a small quantity of china-clay added to keep it in suspension and cause it to form an even coating upon the ware. The ware is coated by being dipped into the liquid. After drying it is heated in an enamelling furnace at 700° to 900°, where the coating becomes fused on to the metal to form on cooling a hard glossy enamel. The semi-transparent enamel must be opacified with some material which is infusible at the temperature of the enamelling furnace and which does not readily form a translucent silicate. Tin oxide is generally used as an opacifier for white enamels, but owing to its high price several substitutes have been introduced, of which the commonest is oxide of antimony. Both trioxide and pentoxide are used, the latter in the form of sodium metantimonate.

Opacifiers can be added to the mixture in the fritting furnace or to the mill in which the finished frit is ground before application to the ware. If added in the fritting furnace the opacifier is exposed to a high temperature for several hours and is more likely to combine with the silicates of the enamel mixture than when added in the mill. Tin oxide, and also other metallic oxides such as those of cobalt, iron and manganese for coloured enamels, are added in the mill, thus avoiding the loss of opacity which might take place if they were fritted with the silicate mixture. When antimony pentoxide, in the form of sodium metantimonate, is used it is added in the mill like other metallic oxides, but antimony trioxide is almost always added in the fritting furnace, as it is found in practice that the results are more satisfactory than if it is added in the mill. This fact is of some importance in determining the final state of oxidation of antimony in the finished enamel. If antimony, either as trioxide or sodium metantimonate, is added in the mill it remains for the most part unaltered in the final enamel. The duration and temperature of heating in the enamelling furnace are not sufficient to cause much oxidation or reduction or other changes in the state of the antimony. When, however, antimony oxides are added in the fritting furnace, with its greater heat and much longer time of fusion, they undergo oxidation or reduction with an apparent tendency to reach a condition of equilibrium between the higher and lower states of oxidation. Thus antimony trioxide is found to have been partly converted into pentoxide, and pentoxide partly into trioxide, with a proportion in the form of tetroxide intermediate between the two. Possibly it is the pentoxide and tetroxide that are the valuable opacifying agents, and the addition of trioxide in the fritting furnace, instead of in the mill as is customary with other metallic oxides, ensures the conversion of much of it into pentoxide. It would seem that approximately half of the trioxide is thus converted into higher oxides in the fritting furnace (see table on p. 219).

Whatever the state of oxidation of antimony in the finished enamel, it cannot be dissolved by the organic acids of food unless the enamel matrix itself, which encloses the particles of antimony oxide, is first dissolved or disintegrated. The extent to which this can take place depends largely upon the silica content of the enamel mixture. Low-silicate ‘soft’ enamels are much more readily dissolved than high-silicate or ‘hard’ enamels, just as with lead silicate glazes in pottery. Hard enamels are more expensive to produce than soft enamels, being less easily fusible and requiring a higher temperature in the enamelling furnace. It is therefore the cheaper classes of ware which are more likely to be coated with soft antimony enamels and which are liable to cause contamination of food. Hollow-ware articles made with seams, such as water-cans and buckets, are also generally coated with a soft enamel, since this runs more readily into the seams. All the poisoning outbreaks from antimony enamel in recent years have been due to the use of seamed ware for preparing or storing lemonade. Some of this ware gave up as much as 17·5 mg. of soluble antimony per square decimetre of surface when treated with hot 2 per cent tartaric acid solution. Among articles made without seams, a pie-dish guaranteed acid-proof gave up 9·5 mg. of tervalent antimony per $dm.^2$ under similar conditions of treatment (see table on p. 219).

Coste and Garratt (1935) determine the suitability of enamelled hollow-ware vessels for preparing or storing food by filling them with a boiling 0·5 per cent solution of citric acid and allowing them to stand for 24 hours. On evaporation of the solution and ignition of the residue the amount of ash should not exceed 1 mg. per $cm.^2$ of enamelled surface. On repetition of the treatment with a similar volume of the boiling acid solution the ash must not exceed 0·5 mg. per $cm.^2$ of surface. In neither test must the ash contain more than traces of antimony.

There is perhaps no serious objection on health grounds to the incorporation of antimony oxide in hard enamels, or to the use in the mill of the pentoxide or sodium antimonate, but in view of the practical difficulties of control it might be more satisfactory to prohibit altogether the use of antimony compounds in certain classes of enamelled vessels. This question is fully discussed in the Ministry of Health report referred to above. No regulations on the use of antimony in enamels have yet been made in this country.

ANTIMONY CONTAMINATION OF FOOD FROM OTHER SOURCES. Manley (1930) found 17 p.p.m. of antimony in processed Gruyère cheese which had been wrapped in tin-foil containing 3·2 per cent of antimony. The object of alloying tin with antimony is to increase the resistance of the tin to corrosion and to facilitate the rolling of the foil. Antimony often appears as a black or brown stain on the surface of the cheese after corrosion and solution of the tin. Solder frequently contains antimony and it is a constituent of various alloys such as pewter and Britannia metal which may come into contact with food. According to Beythien (1936) antimony in this metal is completely insoluble in foods, but corrosion would probably take place on long contact with food in presence of air.

Antimony pentasulphide is a common constituent of rubber tubing and rings. Bleyer and Spiegelberg (1933) found that beer, lemonade and wine could take up considerable amounts of antimony from rubber tubing. 400 ml. of each of these liquors was allowed to remain in contact with 20 g. of the tubing for periods varying from 16 to 69 days. New tubing of good quality gave up 4 to 28 mg. of antimony per 100 g. of rubber, the higher amounts to the more acid liquids. From 100 g. of tubing twenty years old 30 to 326 mg. of antimony were dissolved. Donovan (1934) also found antimony in red rubber hose used for conveying beer from casks. He considered that, although the risk of poisoning might be slight, rubber used for this purpose should not contain antimony.

Antimony has been found in Japanese canned oranges (*Ann. Rept. C.M.O.* 1934). Some of the samples contained 20 p.p.m. (0·14 gr. per lb.) corresponding to 0·39 gr. of tartar emetic, or about half an emetic dose, in one pound. It is not recorded whether the tin-plate or solder of the cans contained antimony. Possibly the oranges had been stored or prepared in enamelled vessels, or the fruit may have been treated on the trees with an insecticide containing antimony. Tartar emetic is an effective insecticide for certain species of fruit flies, and it has been used on citrus trees in the United States (Davidson, Pulley and Cassil 1938).

On one occasion it has actually happened that the contents of a bottle labelled ‘ tartar emetic ’ were used by mistake instead of ‘ cream of tartar ’ for making self-raising flour.

THE DETERMINATION OF ANTIMONY IN FOOD

DESTRUCTION OF ORGANIC MATTER. According to Davidson and others (1938) antimony is not lost when vegetable material is ashed at 550° in a muffle, but in view of the volatility of antimony trichloride it would seem preferable to use a wet oxidation method. Bamford (1934) mixes biological material with sufficient magnesium oxide to give an alkaline reaction and then with 35 to 40 ml. of saturated magnesium nitrate solution for every 100 g. and ignites the mixture to a white ash. Antimony and arsenic are converted by this treatment into non-volatile pyroantimonate and pyro-arsenate respectively.

REDUCTION TO METAL. Antimony is readily reduced to metal and can be precipitated from hydrochloric acid solution as a black powder by zinc, copper, aluminium, nickel, iron and lead. Manley (1930) separates it from cheese by wet oxidation followed by reduction with a zinc-platinum couple. Antimony is deposited on the platinum and can be dissolved off with strong hydrochloric acid and determined by one of the methods indicated below. Evans (1922, 1923) deposits antimony on copper as in the Reinsch test (p. 173) after removing arsenic by precipitation with hypophosphorous acid (p. 201), and Clarke (1928) reduces antimony compounds with iron powder.

VOLUMETRIC DETERMINATION. Antimony trioxide is not oxidised by iodine in acid solution: on the contrary the pentoxide is reduced to trioxide by hydriodic acid, and this reaction can be used for the determination of the pentoxide. In alkaline (sodium bicarbonate) solution antimony trioxide is oxidised to pentoxide by iodine, and this reaction is the basis of the well-known standard method of determining antimony. The trioxide can be titrated with permanganate, dichromate or bromate in acid solution.

The bromate titanium chloride method of Zintl and Wattenberg was applied by Beck and Schmidt (1928) to the determination of tervalent and quinquevalent antimony taken up by food from enamelled ware. For tervalent antimony the solution (about 50 ml.) containing about 10 per cent of hydrochloric acid and a small quantity of methyl orange as indicator, is titrated with 0·001N potassium bromate solution until colourless. 1 ml. of the bromate solution is equivalent to 0·0609 g. of antimony. For total antimony the solution after bromate titration, or if preferred a further portion (about 50 ml.) of the original solution, is first reduced with titanium chloride using phosphotungstic acid as indicator. For this it is heated nearly to boiling-point and treated with ten drops of a 10 per cent solution of phosphotungstic acid. Titanium chloride solution (approximately N/10) is added with constant shaking until a marked blue colour is developed which does not disappear in two minutes. Three drops of a 1 per cent copper sulphate solution are added as a catalyst, and the solution is shaken and exposed to air whereupon the blue colour should disappear. The solution is then titrated with 0·001N potassium bromate using methyl orange as indicator. The amount of quinquevalent antimony is represented by the difference between total and tervalent antimony. The presence of stannic salts does not interfere with the determination, since they are not reduced by titanium chloride.

In alloys of tin and antimony, containing a substantial amount of antimony, this can be determined by permanganate titration (Low 1907). The alloy is heated with concentrated sulphuric acid until completely dissolved, tin forming stannic sulphate and antimony forming antimony trisulphate. The solution is diluted, tartaric acid added to keep antimony in solution and the antimony titrated with standard permanganate solution. Tin may afterwards be determined by reduction to stannous chloride by any of the methods given on pages 152 to 155 and titration with iodine. Antimony may be determined in solder by this method. It may also be applied to foods after wet oxidation of organic matter. For this purpose nitric acid is removed and the sulphuric acid residue treated with tartaric acid and further heated until organic matter is destroyed. Antimony will then be in the lower state of oxidation. Barber (1934) has made the interesting observation that pure lead-tin solder is unacted upon by hot concentrated hydrochloric acid but if it contains as little as 0·01 per cent of antimony it is immediately attacked, lead and tin being dissolved and antimony precipitated as black flakes which eventually dissolve in the acid under the influence of atmospheric oxidation. If this preliminary test shows the presence of antimony, 2 g. of the solder are heated in a flask with 12 g. of concentrated sulphuric acid until dissolved and until the precipitated lead sulphate is white. The mixture is cooled and 15 ml. of water added, followed by 15 ml. of hydrochloric acid. The liquid is boiled for ten minutes to expel arsenic. To the hot solution are added 50 ml. of water and 15 ml. of hydrochloric acid and the solution again boiled to ensure solution of antimony sulphate. A small amount of lead sulphate and sulphur will remain undissolved. The liquid is cooled and filtered quickly with suction to prevent precipitation of too much lead sulphate. The residue is washed with 10 per cent sulphuric acid solution, and the filtrate diluted to 400 ml. and immediately titrated with standard permanganate solution.

COLORIMETRIC DETERMINATION AS SULPHIDE. Evans (1922, 1927) determines small quantities of antimony in metals colorimetrically as the orange-yellow sulphide with the addition of gum arabic to keep the precipitate in suspension. Bamford (1934) applies the sulphide colorimetric method to the determination of antimony in biological material. Antimony must first be separated from iron by precipitation as sulphide in acid solution. Th
sulphide is filtered off and redissolved in hot concentre
hydrochloric acid. The solution is then diluted aetric
antimony again converted into sulphide for cohts the
comparison with standards. Järvinen (1923) p alcohol.
deposition of finely divided sulphur by additi WITH PYRI-

COLORIMETRIC DETERMINAT Antmony forms
DINE AND POTASSIUM IOD with many alkaloids and
highly coloured iodo-antimona

organic bases, analogous to those formed by bismuth, with the general formula BASE HI, METAL I_3. Caille and Viel (1923) used the reaction with antipyrine and potassium iodide as a test for antimony, but Clarke (1928) found that the pyridine compound is more soluble and better suited for quantitative determination. The method as developed by him is intended for the determination of small amounts of antimony in high-grade tin. It is therefore applicable to the examination of tin-foil used for wrapping cheese and other foods and also, with the necessary modifications, to the determination of traces of antimony in foods. The metal is dissolved in concentrated hydrochloric acid containing bromine, and oxalic acid added to keep tin in solution on subsequent dilution. Antimony is precipitated from the solution with metallic copper and the deposit stripped with sodium peroxide. The small amount of copper which comes off with the antimony is precipitated in alkaline solution with hydrogen sulphide and the solution filtered. Antimony remains in solution and is subsequently converted into sulphate and added to a solution containing gum arabic, potassium iodide, pyridine, sulphur dioxide and dilute sulphuric acid. The resulting clear yellow solution is then compared with standards. Full details of the method are given in Clarke's paper.

OTHER METHODS. The behaviour of antimony in the Marsh and Gutzeit methods is referred to on pages 191, 193. Sanger and Gibson (1907) and Grant (1928) have studied the determination of small quantities of antimony by the Marsh method and their papers should be consulted for details. The chief requirements are that only small amounts of antimony should be present and that the stibine should be quickly removed from the cathode and carried forward by a rapid stream of hydrogen from a separate generator ; otherwise most of the antimony is deposited as metal on the cathode instead of being evolved as stibine. Whether this is due, as suggested by Grant, to decomposition of the stibine as soon as it is formed, or whether the first stage is reduction to metallic antimony, further reduction taking place only in a rapid stream of hydrogen, is not clear. Davidson and others (1938) use the Gutzeit method for the examination of spray residues when tartar emetic has been used as an insecticide. They state that it is suitable for 0·025 to 0·150 mg. of antimony (cf. p. 194). It is not necessary to reduce quinquevalent to tervalent antimony before testing. Tananaeff and Ponomarjeff (1935) find that when antimony solutions are reduced with tin all the antimony separates in the metallic state and no stibine is evolved, so that with tin and hydrochloric acid arsenic can be determined in presence of antimony. Wilson (1940) uses a tin-platinum couple for this purpose and finds that this method gives the best separation of antimony and arsenic in micro-analytical work. Antimony can also be precipitated with 8-hydroxyquinoline ('oxine') from ammoniacal acetate solution or from tartrate solution, the precipitates differing slightly in composition according to the medium (Pirtea 1939).

Goodwin and Page (1943) point out that the polarographic method (p. 53) is the only one which will distinguish between ter- and quinquevalent antimony in biological materials. They have applied this method to the investigation of the excretion of antimony after administration of organic antimonials. Page and Robinson (1942) have also used it for the determination of antimony and bismuth when present together in biological material.

EXAMINATION OF ANTIMONY ENAMELS AND HOLLOW-WARE. Antimony may be present in enamels in three stages of oxidation, Sb_2O_3, Sb_2O_4 and Sb_2O_5. The constitution of the intermediate tetroxide is doubtful: analytically it can be treated as a mixture of the higher and lower oxides. The following is an outline of the methods used for the determination of the three oxides in hollow-ware enamels opacified with antimony. For fuller details the Ministry of Health report on this subject (Monier-Williams 1934) should be consulted.

The enamel, ground to an impalpable powder, is thoroughly extracted with 2N hydrochloric acid solution at room temperature. This dissolves out antimony trioxide which is then determined by titration in the presence of bicarbonate with standard iodine solution. To the residual solids are added hydrochloric acid and potassium iodide and the mixture is distilled. Antimony pentoxide in the enamel is reduced to trioxide by this treatment and an equivalent amount of iodine is liberated and passes over into the distillate. The iodine in the distillate is then titrated with thiosulphate, and the antimony trioxide remaining in the flask is also titrated with iodine after adding bicarbonate. If no tetroxide is present in the enamel these two titrations should agree and should furnish a measure of the amount of pentoxide in the enamel. If tetroxide is present the titration figure of the residual trioxide will be higher than that of the iodine in the distillate by an amount corresponding to half of the tetroxide. The difference between the titrations, multiplied by two, will represent the amount of tetroxide present, and a corresponding deduction must be made from the pentoxide figure. The method of calculation is based on the assumption that the tetroxide behaves, on distillation with hydriodic acid, as an equimolecular mixture of trioxide and pentoxide.

The sum of the antimony present as trioxide, tetroxide and pentoxide gives the total antimony. This figure may be checked by fusing 0·25 to 0·5 g. of the powdered enamel with three times its weight of sodium hydroxide in a silver crucible. The melt is dissolved in water and the solution made strongly acid with hydrochloric acid. 0·2 g. of potassium iodide is added and the iodine liberated is removed by distillation. The antimony in the residue is determined by titration in alkaline solution with N/100 iodine solution.

The resistance of an antimony enamel to attack by organic acids may be examined by pouring into the vessel a boiling 2 per cent solution of tartaric acid and allowing it to stand for half an hour with occasional stirring. The solution is then poured off and, if evaporation has taken place, it is made up to its former volume with water. Antimony in the solution is then determined by methods similar to those given above for enamels.

The accompanying table, taken from the Ministry of Health report on this subject (1934), shows the antimony content of a number of commercial enamels and their resistance to treatment with tartaric acid solution.

Number of Sample	Description	Antimony extracted from vessels by hot 2 per cent tartaric acid solution				Antimony in the enamel chipped from the vessels				
		Mg. per litre		Mg. per dm.^{2} of surface		Sb^{III} per cent	Sb^{IV} per cent	Sb^{V} per cent	Total Sb per cent. Sum of Sb^{III}, Sb^{IV} and Sb^{V}	Total Sb per cent. by fusion with NaOH
		Sb^{III}	Total Sb	Sb^{III}	Total Sb					
1	Small jug . . .	nil	nil	nil	nil	0·39	nil	0·58	0·97	0·97
2	,, ,, . . .	3·62	6·26	0·65	1·12	0·80	0·07	0·87	1·74	—
3	Large jug (seamed)	138·0	154·3	15·65	17·49	1·78	0·84	2·10	4·72	4·39
4	Bucket (,,)	3·0	3·24	0·40	0·44	0·23	0·11	0·50	0·84	0·88
5	Chamber . . .	61·8	69·0	12·93	14·43	1·12	0·15	0·91	2·18	2·24
6	,, . . .	60·0	66·0	11·63	12·8	1·11	0·04	0·85	2·00	1·99
7	Pie dish . . .	79·2	87·3	9·45	10·41	1·34	0·17	2·05	3·56	3·12
8	Mug	0·43	6·86	0·08	1·23	0·18	0·04	1·19	1·41	1·56
9	,, ...	0·32	1·67	0·06	0·30	0·13	nil	1·65	1·78	1·60
10	Large jug (seamed)	—	—	—	—	2·96	1·22	2·28	6·46	6·30
11	Bucket (,,)	—	—	—	—	3·75	1·22	0·93	5·90	5·69

Sample 1 had an undercoat containing antimony, but no antimony in the outer enamel.

Sample 3 was one of a batch that caused an outbreak of poisoning in 1932.

Sample 7 was from makers who stated that their enamel was hard, and expressed themselves confident that it did not yield soluble antimony to foods.

Samples 8 and 9 were enamelled with a sodium metantimonate enamel.

Samples 10 and 11 were specimens of enamels taken from vessels which had caused outbreaks of poisoning in 1928 and 1929.

For the detection and determination of very small amounts of antimony in enamels the most trustworthy method is precipitation with hydrogen sulphide in presence of oxalic acid which prevents the precipitation of tin. The finely powdered enamel must first be fused with three times its weight of sodium hydroxide in a silver crucible, the melt dissolved and the solution acidified with hydrochloric acid. 0·1N iodine solution is added to oxidise any tin present and excess of iodine is removed by careful addition of sodium thiosulphate solution. About 5 per cent of finely powdered oxalic acid is dissolved in the liquid by shaking, and the solution is saturated with hydrogen sulphide and allowed to stand overnight. Antimony in the precipitate is then determined by any of the standard methods. If traces only of antimony are present they are indicated by an orange ring at the end of the glass tube through which hydrogen sulphide is led into the liquid.

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7

[SELENIUM]

SELENIUM has a toxic effect upon animals comparable with, and perhaps even greater than, that of arsenic, giving rise to somewhat similar symptoms. In 1901 evidence was given before the Royal Commission on Arsenical Poisoning, suggesting that some of the cases of illness attributed to arsenic in beer (p. 164) were in fact due to selenium, or at any rate that selenium was a contributory cause. The witness in question stated that he had found considerable quantities of selenium in sulphuric acid made from seleniferous pyrites and as much as 6 p.p.m. in two samples of beer.

The suggestion was examined in some detail by the Commission who, without expressing any opinion on the question of the toxicity of selenium, drew attention to the complete lack of any confirmatory evidence that selenium had been found in appreciable amounts in beer or brewing materials.

After this, little was heard of selenium poisoning until 1933–4, when it again came into prominence as the cause of the so-called ‘ alkali disease ’ or ‘ blind staggers ’ of horses, cattle and pigs in South Dakota, Wyoming, Nebraska and neighbouring states.

In the acute form of the disease, ‘ blind staggers ’, prevalent in Wyoming, the animals become blind and suffer from muscular weakness and apathy, accompanied by congestion of the lungs, haemorrhage and convulsions. In the chronic form, ‘ alkali disease ’, predominant in South Dakota, the symptoms are wasting, loss of hair from mane and tail and a characteristic inflammation and deformity of the hoofs.

The disease was formerly thought to be due to excessive alkalinity in the soil, but Franke (1934) showed that it was caused by a toxic substance in grain grown in these districts and identified this as selenium, which Nelson, Hurd-Karrer and Robinson (1933) had already found to be absorbed by wheat and other crops from soil and from insecticidal spray residues and to be extremely toxic to laboratory animals.

The contamination of crops with selenium over such wide areas is a serious agricultural problem in certain parts of the United States. So far it is only in animals that the occurrence of selenium poisoning has been fully proved, but there is reason to suppose that human beings living in seleniferous areas are not completely immune, and that some degree of chronic poisoning may in fact exist in certain districts.

SELENIUM IN SOIL. Selenium occurs generally in soils derived from cretaceous pyritic shales, especially in districts with a low rainfall. It exists in these soils chiefly as basic ferric selenite, $Fe_2(OH)_4SeO_3$, calcium selenate, $CaSeO_4$, and organic selenium compounds derived from decayed plant tissue (Williams and Byers 1936). The amount of selenium in the soils of the affected districts ranges from traces up to 12 p.p.m., with occasional soils containing as much as 30 p.p.m. The average amount in 122 soil samples taken from one square mile in South Dakota was 4·8 p.p.m., but in most localities it is less than this (1 to 3 p.p.m.). Selenium is removed from the soil by irrigation, and toxic soils are found only in dry climates. They are not confined to the United States, but occur in Mexico, Argentina, Chile, Peru, China and possibly in other countries.

ABSORPTION OF SELENIUM BY PLANTS. Arsenic, selenium, tellurium, molybdenum and vanadium form a highly toxic group of elements. Of these arsenic is not absorbed by plants and tellurium has been found only in a few native plants in Wyoming. Selenium, molybdenum and vanadium are all absorbed by plants, but only the two first-named have so far been found to be taken up in amount sufficient to poison animals.

Plants may be divided roughly into three classes according to their capacity for taking up selenium (Miller and Byers 1937):

1. Those which absorb it readily and grow best on seleniferous soils. There are about thirty of these, mostly perennial plants of the orders *Leguminosae*, *Cruciferae* and *Compositae*, in particular *Astragalus*, *Aster*, *Oonopsis* and *Stanleya* (Trelease 1938).
2. Plants which can absorb moderate or even large amounts without severe injury (wheat, barley, oats, maize, rye, onions).
3. Plants showing a limited tolerance for selenium and absorbing only slight amounts (up to 5 p.p.m.) when grown on seleniferous soils. To this class belong many grasses and also most garden vegetables.

Plants of the first of these classes seem to thrive only upon soil containing selenium and absorb relatively enormous amounts of it. Some species of vetch (*Astragalus*) can take up over 4,000 p.p.m., and very large amounts have been found also in *Oonopsis* and *Stanleya* (Byers and others 1935, 1936, 1938). They have been called 'indicator plants' since their presence in an area is an indication that the soil is seleniferous.

Most plants cannot take up selenium from inorganic compounds, but 'indicator' plants are able to do so and can convert the selenium into a form easily absorbed by crops and pasture. Their growth and decay over a long period results therefore in a gradual accumulation in the soil of organic selenium assimilable by other plants. For this reason the indicator plants are also termed 'converter' plants. Very large amounts of selenium in soil (80 p.p.m.) are toxic even to these plants, but actually such amounts are rarely met with.

The second class includes grain crops, and it is these which are mainly responsible for selenium poisoning in animals. Cereals will grow normally in soils containing up to about 5 p.p.m. of selenium and may take up as much as 30 p.p.m. (Robinson 1936). Wheat grown on soils containing 0·5 to 1 p.p.m. of selenium may take up enough to become toxic to animals. Smith and Westfall (1937) give the following figures for the selenium content of cereals grown in seleniferous areas: wheat 1·1 to 18·8 p.p.m., maize 1·0 to 14·9, barley 1·6 to 5·7, oats 2·0 to 10·0 and rye 0·9 to 3·8 p.p.m.

Among the many factors influencing the absorption of selenium by plants, particularly cereals, is the sulphate content of the soil. Selenium shows a close analogy chemically with sulphur, and the selenium analogues of many organic sulphur compounds have been prepared. The absorption of selenates by wheat varies inversely as the concentration of sulphate in the soil. The higher the sulphate content the less toxic is the soil to wheat, and the greater the amount of sulphate taken up by wheat the less that of selenium. Free sulphur in the soil has a similar effect.

Selenium in wheat is associated with the gluten. It has been suggested that it may partially replace the sulphur of cystine and methionine (Painter and Franke 1936), but it is probably more loosely combined. Westfall and Smith (1939) say that it can all be removed by treatment of gluten with bromine in hydrobromic acid or with hydrogen peroxide, without marked disintegration of the protein. Part of the selenium in proteins appears to be present in a form readily split off by alkalies (Franke and Painter 1936).

Plants of the third class, which includes grasses, garden vegetables, etc., contain for the most part much smaller amounts of selenium, with a few exceptions such as onions. Smith and Westfall (1937) give the following figures for some of these plants:

Beets	0 to 1·2 p.p.m.	Onions	0·4 to 17·8 p.p.m.
Beans	0·4 to 2·0 „	Potatoes	0·2 to 0·9 „
Carrots	0·4 to 1·3 „	Peas	0·4 to 2·0 „
Cabbage	0·4 to 4·5 „	Tomatoes	0 to 1·2 „

As a general rule it seems to be accepted that any soil containing more than 0·5 p.p.m. of selenium is potentially dangerous and also any vegetation containing more than 4 p.p.m. In South Dakota, on soil averaging 4·8 p.p.m., the average amount of selenium in all vegetation including indicator plants was found to be 36 p.p.m.

The high selenium content of wheat in these districts has naturally raised the question whether grain from any other parts of the world may be affected. Robinson (1936) examined wheat from New South Wales, Argentina, Mexico, Canada, South Africa, Spain, New Zealand and Hungary, as well as from non-seleniferous areas in the United States, and found from 0·1 to 1·9 p.p.m. of selenium. Thorvaldson and Johnson (1940) examined 230 composite samples of Saskatchewan wheat, representing 2,230 individual samples, and found a maximum selenium content of 1·5 p.p.m. and an average of less than 0·5 p.p.m. Among individual wheat samples one contained 4 p.p.m. and no others were above 3 p.p.m.

TOXICITY OF SELENIUM. For small animals (cats, rabbits, rats, etc.) the limit for chronic poisoning appears to be about 0·5 mg. per kilogram of body weight, but the daily ingestion of about 0·2 mg. per kilogram may cause disturbance of digestion and liver function. Miller and Williams (1940) give the minimum lethal doses (in mg. per kilogram body weight) as 3 for horses, 9 for cattle, and about 12 for pigs. Grain containing 10 p.p.m. of selenium produces typical ‘ alkali disease ’ in pigs (Schoening 1936) and 24 p.p.m. kills them. Prolonged feeding with seleniferous food causes loss of reproductive powers (Franke and Potter 1936). The toxicity of food selenium may be greatly influenced by dietary factors, so that a level of intake which under certain conditions of diet may be relatively harmless can become very toxic under other conditions (Smith 1939). Possibly this may depend in some degree upon the extent to which selenium is reduced to the metallic state in the alimentary tract, since metallic selenium, owing to its insolubility, would be relatively non-toxic. The curious observation has been made that arsenic (5 p.p.m. in water) protects rats from poisoning by selenium, both organic and inorganic, but after prolonged feeding of the animals for a month or more on seleniferous food arsenic sometimes loses its efficacy. Dogs have been kept quite healthy for 12 months on a diet containing 12 p.p.m. of selenium if given 5 p.p.m. of arsenic in their drinking water : without the arsenic they all died (Moxon 1938, Moxon and Dubois 1939).

Under ordinary conditions the limit of toxicity to animals, and probably also to men, of selenium in food is placed at about 3 to 4 p.p.m. (Munsell and others 1936). This amount is readily exceeded in grain but not so easily in the mixed diet of human beings. This difference of diet between human beings and animals is probably the reason why the former are apparently little affected by selenium in districts characterised by widespread poisoning of horses and cattle. Cereals, of course, enter largely into the human dietary but much of the selenium in wheat is eliminated in the bran. Other human foods are low in selenium whereas horses and cattle not only eat large quantities of cereal by-products such as bran but can hardly avoid picking up highly seleniferous plants when grazing. The cooking of vegetables for human food may also be an important factor since much of the selenium is removed by treatment with water (Beath and others 1937). Another point of possible significance is that protein diminishes the toxicity of selenium and that it is not so poisonous in high-protein foods as in high-carbohydrate foods (Smith 1939). Casein has been found to have a high protective action in rats. 30 per cent of casein in the food neutralises the toxic effect of 35 p.p.m. of selenium (Gortner 1940). Lactalbumin and ovalbumin are also effective and, to a much less extent, wheat gluten and gelatin (Smith 1941).

Eggs are liable to contain selenium derived from poultry food (Poley and others 1937). Smith and Westfall (1937) give fairly high values for eggs (0·25 to 9·1 p.p.m.) and for meat (1·2 to 8 p.p.m.) in seleniferous districts, but unexpectedly low values for bread (0·25 to 1 p.p.m.). Milk contained 0·2 to 1·3 p.p.m. These variations are so wide that it is difficult to draw conclusions from them as to the average selenium content of a human dietary in seleniferous areas. It would appear that people in these districts may be exposed to the risk of ingesting from 0·5 to 10 mg., or even more, of selenium daily and that they sometimes excrete it in their urine in amounts comparable with those found in the urine of animals suffering from poisoning. Excretion of 0·2 to

0·5 p.p.m. in human urine is common and 2 p.p.m. has been recorded by Smith and Westfall (1937). Normal urine from a non-seleniferous area has shown an average of 0·04 p.p.m. (Sterner and Lidfeldt 1941). This was in an area in which the selenium content of wheat products as consumed ranged from 0·26 to 0·39 p.p.m. and there was no other known source of selenium. Selenium exists in the urine as an ether-soluble compound. A good deal of vague ill-health, indigestion, pallor, malnutrition and nervous symptoms and also a particular form of dermatitis have been found to be associated with seleniferous areas, but no illness which can be ascribed with certainty to selenium. The presence of methyl selenide in the breath, making it smell of garlic, is one of the early symptoms of selenium poisoning, and this may occur with a daily ingestion of a few milligrams. It is probable that continued ingestion of 5 to 10 mg. daily would lead to nervous symptoms and gastro-intestinal disorders. Some of the ingested selenium is stored as a protein compound in the tissues (Smith, Westfall and Stohlmann 1938).

There are no records of selenium poisoning from food in this country other than that alleged, without sufficient evidence, to have been due to selenium in beer in 1900. Certain parcels of barley imported from the United States about twenty years ago proved to be highly poisonous to pigs, but this was attributed to infection of the barley with toxic moulds, and the possibility that it might have been due wholly or partly to selenium does not appear to have been suspected.

SELENIUM INSECTICIDES. The selenium analogues of alkaline polysulphides are more effective against red spider than are the sulphides themselves (Gnadinger 1933). An insecticide called ‘ Selocide ’ is made by dissolving selenium in the double sulphide of ammonium and potassium and has the formula $(KNH_4S)_5Se$ (Hoskins 1938). Citrus fruits and grapes sprayed with this substance contain small amounts of selenium, up to 2 p.p.m. in the rind of oranges and rather more in grapes. The repeated use of these insecticides might eventually result in the soil becoming seriously contaminated with selenium (cf. lead arsenate, pp. 81, 168).

With lead arsenate soil contamination is of no great consequence since neither lead nor arsenic are taken up by plants from the soil, but with selenium there might be a danger, perhaps only in dry climates, that it would be absorbed by succeeding crops in amount sufficient to make them actively toxic.

It has been suggested that small quantities of selenium compounds, below the limit of tolerance for cotton plants, should be added to the soil in cotton-growing districts, the idea being that the plants will take up enough selenium to become toxic to certain insect pests although not enough to affect the growth of the plants (Mason and Phillis 1937). Selenium in amounts of 50 to 100 p.p.m. in the foliage of different plants has been found effective against certain insects (aphis and red spider) but its action appears to be highly selective and some species of beetles are not poisoned by *Astragalus* seeds containing 1,475 p.p.m. (Trelease and Trelease 1937).

THE DETERMINATION OF SELENIUM IN FOOD

SEPARATION FROM ORGANIC MATTER. It should be possible to destroy organic matter completely without loss of selenium by the ordinary process of wet oxidation with sulphuric and nitric acids, provided that oxidising conditions are maintained throughout. Some difficulty, however, has been experienced in preventing loss of selenium by volatilisation even when excess of nitric acid has been used. Several analysts have recorded serious losses if the digestion is carried out too quickly, with too copious an evolution of gas, or if the temperature of digestion is too high. They have therefore recommended partial destruction of organic matter by digestion with sulphuric and nitric acids at a low temperature, followed by distillation of selenium as bromide. The probability is that oxidising conditions have not always been maintained throughout the digestion and that selenium, which is very easily reduced, may form volatile compounds if reducing conditions occur at any stage in the digestion.

Pending further information on the behaviour of selenium under ordinary conditions of wet oxidation with complete destruction of organic matter, it seems better to adopt the official method of the Association of Official Agricultural Chemists—partial digestion with the addition of mercuric oxide followed by distillation as bromide. Mercuric oxide, in addition to catalysing the reaction, prevents volatilisation of selenium, possibly by keeping it oxidised or by forming mercuric selenide. Curl and Osborn (1938) consider its presence essential.

About 5 to 10 g. (dry weight) of the sample are mixed in a 600 ml. Pyrex beaker or Kjeldahl flask with 0·5 g. of mercuric oxide and treated with a cooled mixture of 50 ml. of sulphuric acid and a volume of nitric acid equal to 10 ml. per gramme of the sample taken. The whole is allowed to stand for 30 minutes and is then heated *gently* until nitrous fumes are no longer evolved and the liquid becomes dark brown. It is cooled, diluted with 25 ml. of water, cooled again and 50 to 60 ml. of 48 per cent hydrobromic acid containing 0·5 per cent by volume of free bromine are added. The flask is attached to an all-glass distilling apparatus provided with a thermometer and distilled until the temperature reaches 130°, the receiver being kept cool. If the distillate contains waxy or insoluble matter, as it generally does, it is filtered through asbestos.

DETERMINATION OF SELENIUM. The selenium in the distillate can be determined in four ways, turbidimetric, volumetric, gravimetric or colorimetric.

(*a*) *Turbidimetric Determination.* The distillate containing selenium is saturated with sulphur dioxide, 0·1 to 0·5 g. of hydroxylamine hydrochloride is added, and the liquid warmed on a steam bath to 80° for 15 minutes. It is cooled and filtered through an asbestos pad in a Gooch filter, or through a sintered glass filter, and the residue washed. The precipitated selenium is then dissolved off the filter with 10 ml. of 48 per cent hydrobromic acid containing about 1 per cent of free bromine, the selenium in the filtrate re-precipitated with sulphur dioxide and hydroxylamine, and the pink turbidity compared with that of standards prepared in the same way. Dudley and Byers (1935) recommend the addition of gum arabic to stabilise the turbidity (cf. also Robinson 1933).

The method is suitable for amounts of selenium between 0·01 and 0·5 mg. By comparing the turbidities in a beam of light in a dark room the delicacy can be increased. It is subject to errors of ±15 per cent (Matthews and others 1937).

(*b*) *Volumetric Determination.* Selenious acid oxidises hydriodic acid to free iodine which can be titrated with thiosulphate. The precipitated selenium in the solution is apt to obscure the end-point and also to absorb iodine, but Werner (1940) claims that the interference is negligible and that the end-point is very sharp.

An alternative method is one in which selenious acid is allowed to react with sodium thiosulphate in hydrobromic acid solution to form sodium tetrathionate and selenotetrathionate, and the excess of thiosulphate is then titrated with standard iodine solution,

$$H_2SeO_3 + 4Na_2S_2O_3 + 4HBr = Na_2S_4SeO_6 + Na_2S_4O_6 + 4NaBr + 3H_2O.$$

The selenium, after precipitation from the distillate as in (*a*) above, is dissolved in 1 to 2 ml. of 48 per cent hydrobromic acid containing 1 per cent of bromine and the solution transferred with washings to a 30- or 50-ml. beaker, keeping the total volume to about 10 ml. A strong solution of sulphurous acid is added until the colour due to bromine almost disappears, and the last traces of bromine are removed with one or two drops of 5 per cent phenol solution. Removal of most of the bromine with sulphurous acid avoids the production of a heavy precipitate of tribromophenol. A moderate excess of very dilute (0·0005 to 0·01N) standard thiosulphate is added and the liquid titrated with standard iodine solution with starch as indicator, using a microburette. Standards must be prepared and titrated in exactly the same way, care being taken that the volume of liquid titrated is as far as possible the same in all cases, otherwise with very dilute solutions the dilution error of the titration may be considerable. 1 ml. of 0·001N sodium thiosulphate is equivalent to 0·02 mg. of selenium (*J. Assoc. Off. Agric. Chem.*, 1939, **22**, 85). Coleman and McCrosky (1937) give a slightly different version of this method (cf. also Wernimont and Hopkinson 1940, Klein 1941).

Another method given by Coleman and McCrosky (1937), in which selenium is oxidised to selenious acid by iodic acid in hydrochloric acid solution with liberation of iodine, which is then oxidised to iodine monochloride by iodic acid, does not seem to have been applied to the determination of traces of selenium in plants or foods.

(*c*) *Gravimetric Determination.* The gravimetric method is suitable only for quantities of selenium of 1 mg. or more. Selenium, after precipitation from the distillate as in (*a*) above, is dissolved in 30 ml. of hydrobromic acid containing 1 per cent of bromine and the filter washed with 30 ml. of water. The selenium in the filtrate is re-precipitated with sulphur dioxide and hydroxylamine hydrochloride, as in the turbidimetric method, and the liquid heated and allowed to stand for 48 hours. The precipitate is then separated by filtration on asbestos in a weighed Gooch crucible, washed, and dried at 85°–90° (Williams 1937).

(*d*) *Colorimetric Determination.* This method is based on the blue colour given by codeine with a solution of selenious acid in concentrated sulphuric acid. The fact that codeine and morphine give a blue to green colour with sulphuric acid containing traces of oxidising agents has been known for a long time. The reaction is more delicate with selenious acid than with other oxidising agents, and has been used by Mecke (1899) as a test for morphine and codeine and by Schmidt (1914) to detect traces of selenious acid in sulphuric acid. The colour is given by as little as 0·5 p.p.m. of selenium in the concentrated acid, whereas the sulphite test (precipitation of red selenium) has a delicacy of only 20 p.p.m. Schmidt found that ferric iron also produced a blue colour and interfered with the test, but only if present in comparatively large amount. Horn (1934) found that 2 g. of ferric sulphate digested with 50 ml. of concentrated sulphuric acid and mercuric oxide for one hour gave no colour with codeine. Possibly all the iron was removed, together with the mercury, by precipitation as anhydrous sulphate. Horn tried the effect of traces of all the commoner elements digested with concentrated sulphuric acid and found that under these conditions none of them gave any colour with codeine except vanadium, which gave a blue colour similar to that given by selenium. Molybdenum gave no colour, although Froede's reagent (molybdic acid in sulphuric acid) is known to give molybdenum blue with codeine. Tellurium also gave no colour, although Schmidt had found that the liquid became blue after long standing. The codeine test appears, therefore, to be almost specific for selenium in the residue obtained by destruction of plant and animal tissues with sulphuric acid and mercuric oxide. Vanadium alone interferes, but must be present in amount about ten times that of selenium to give a similar colour. The test can therefore be carried out on the sulphuric acid residue obtained by acid digestion, provided that no vanadium is present, thus avoiding the necessity of distilling. Nitric acid must be absent, as it gives a red colour with codeine.

Davidson (1939) and Gortner and Howard (1939) independently developed the codeine test for the determination of selenium in foods and biological material. Since the presence of nitric acid, even in traces, is inadmissible, Gortner and Howard used a wet oxidation process with sulphuric acid, hydrogen peroxide and mercuric oxide, while Davidson used sulphuric acid and mercuric oxide only. There seems to be some difference of opinion as to the effect of traces of iron on the colour reaction. As stated above, iron may give a blue colour, but only if present in comparatively large amount. According to Davidson the presence of iron is essential to the proper development and stability of the colour, and it must be added if the sample does not contain enough. Gortner and Howard do not make any reference to the presence of iron, and Klein (1941) states that both ferrous and ferric iron must be absent, since ferrous iron reduces selenious acid to selenium and prevents its reaction with codeine, and ferric iron itself gives a blue colour with codeine and may lead to high results. Probably traces of iron of the order of a milligram or so are required to catalyse the reaction, but an excess of iron will interfere by producing a blue colour with codeine. Davidson finds that the colour is also affected to some extent by phosphates in the ash, so that it is necessary to add phosphate to the standards to compensate for this.

Davidson's method is as follows: From 2 to 10 g. of plant material are digested with 25 to 75 ml. of sulphuric acid and 0·7 g. of mercuric oxide for 2½ to 3½ hours, when the residual sulphuric acid should be colourless. The mercuric oxide should prevent loss of selenium by volatilisation, but it is advisable to digest the standard solutions for comparison in a similar way and for the same time, so as to compensate as far as possible for any loss of selenium. The standards should receive an addition of 1 ml. of 0·5 per cent ferrous sulphate solution and 1 ml. of a molecular solution of mono- or dipotassium phosphate. When digestion is completed the residual contents of the flasks are made up to a definite volume of about 30 ml. with concentrated sulphuric acid, and allowed to stand overnight in closed vessels to ensure complete precipitation of mercuric sulphate. Aliquot parts (20 ml.) of the clear liquids are then transferred with pipettes to small stoppered flasks or tubes, and treated with 5 to 6 drops of a 2 per cent aqueous solution of codeine sulphate, or of codeine in sulphuric acid, slowly and with constant shaking. The flasks are closed and allowed to stand for about two hours and the colours compared. If more than 0·2 mg. of selenium is present more codeine must be added. The amount of codeine should not be less than 30 times the quantity of selenium present, otherwise the colour may be green instead of blue.

BEHAVIOUR OF SELENIUM IN THE MARSH-BERZELIUS AND GUTZEIT TESTS. Selenium can be reduced to hydride in an electrolytic cell with lead cathode, but not so readily as arsenic, since most of it is converted into elemental selenium and remains in the liquid as an insoluble precipitate (Meunier 1916). The small amount of selenium hydride which is formed is completely retained by lead acetate paper and cannot therefore interfere with the determination of arsenic. If lead acetate paper is omitted selenium can be detected by both the Marsh and Gutzeit methods, but only if present in fair amount. Thus 0·1 mg. of selenious acid gives hardly any stain on mercuric bromide paper and no mirror of selenium in the Marsh test, using an electrolytic cell with lead cathode (p. 180). 0·5 mg. of selenious acid still gives no mirror, but produces a heavy brownish-black stain on mercuric bromide paper. 1 mg. of selenious acid gives a red to orange mirror of elemental selenium at the same distance from the flame as an arsenic mirror and corresponding roughly in intensity with that given by 0·02 mg. of arsenic. Much of the selenium hydride passes on undecomposed and can be detected with lead acetate paper at the outlet of the Marsh-Berzelius tube.

Selenium is deposited upon copper foil from hydrochloric acid solution on heating as in the Reinsch test for arsenic (Evans 1939).

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8

[IRON]

IRON is not usually regarded as a trace element in the body since it forms over 0·004 per cent of the total weight, and the body of an adult contains therefore between 3 and 4 g. The iron content of most foods is, however, very small, and iron seems to have equal claims with zinc and manganese to be classed as one of the trace elements in food.

HAEMOGLOBIN. Haemoglobin contains 0·335 per cent of iron and itself constitutes about 15 per cent of male human blood (rather less in women). A man has therefore roughly 50 mg. of iron in 100 ml. of his blood (500 p.p.m.). On the estimate that the blood constitutes about 8 per cent of the body weight the total quantity of iron in the blood of an adult is from 2·5 to 3·0 g.

Haemin, the parent substance of haemoglobin, has the empirical formula $C_{34}H_{32}O_4N_4FeCl$, and is essentially a compound of ferric iron with four substituted pyrrol rings—an iron porphyrin. It is the hydrochloride of a base, haematin or haem, which according to conditions can exist either as ferric haem (oxyhaematin) or as ferrous haem (reduced haematin). Ferrous haem can combine with a variety of nitrogenous compounds to form different haemo-chromogens, which are stable only in presence of a reducing agent. It can also combine with the protein globin to form haemoglobin, a substance of much higher molecular weight than the haemo-chromogens. Haemoglobin acts as an oxygen carrier in the blood, forming alternately oxyhaemoglobin and reduced haemoglobin. The iron in both of these forms is in the ferrous state (ferrohaemoglobin) in contrast to methaemoglobin (ferrihaemoglobin) in which it is in the ferric state. According to Ramsay (1944) there are indications that about 50 per cent of human blood samples contain significant amounts of methaemoglobin, which is unable to combine with oxygen until it has been reduced. Methaemoglobin is formed if from any cause the red cells are ruptured and haemoglobin escapes into the plasma.

Practically the whole of the haemoglobin of blood is contained in the red corpuscles or erythrocytes, which form about 45 per cent of the total blood volume. Thus the corpuscles themselves contain over 30 per cent of haemoglobin. The red corpuscles are believed to originate as immature cells in the bone marrow. They pass into the main circulation where they develop into mature erythrocytes. During the stages of their development in the bone marrow, and finally in the main circulation, haemoglobin is synthesised and gradually accumulates in them.

Iron is absorbed from food in the intestinal tract direct into the blood stream, apparently in the form of ferrous iron. Ferric salts are particularly prone to form insoluble complexes with phosphoproteins, etc., in which condition they are not dialysable and are not absorbed. Schultze (1940) suggests that only ferrous salts can cross the intestinal barrier, and that the absorption of ferric iron depends upon its reduction in the alimentary tract. Tompsett (1940) has shown that several foods—milk, wholemeal flour, egg-white, etc.—are capable of reducing ferric iron rapidly in acid solution, and suggests that this probably occurs in the stomach. Such reduction would depend upon the solubility of the ferric complex in question.

After absorption into the blood plasma, ferrous iron becomes available for the synthesis of haematin and haemoglobin by the maturing red corpuscles, being apparently first oxidised to ferric iron. Blood plasma contains about 1·2 p.p.m. of this serum or plasma iron in organic combination, representing an intermediate or transport stage between the ferrous iron as absorbed from the intestine and the completed haemoglobin of the erythrocytes.

The haemoglobin of the blood, after carrying out its function of oxygenation for a certain time, is broken down to other bodies and replaced by freshly synthesised haemoglobin. About 5 to 10 per cent of the iron in the erythrocytes is present in the form of intermediate compounds resulting from the breakdown of haemoglobin to the bile pigment bilirubin which itself contains no iron. The iron of these intermediate products of haemoglobin breakdown is collected in the liver, and is practically all used again in the synthesis of fresh haemoglobin. Callender (1945) estimates that 0·83 per cent, or 7 g., of the total haemoglobin of the blood is thus broken down and reformed every day, giving an average life of 120 days for the erythrocytes.

Iron in the liver exists partly in the form of ' ferritin ', a crystallisable iron phosphoprotein containing 20 to 24 per cent of ferric iron (Laufberger 1937). This is apparently the form in which iron is stored in the body.

TISSUE IRON. The blood contains about 70 per cent of the total iron in the body, the other 30 per cent being located in the tissues in various forms, partly as haematin iron and partly as non-haematin iron. Among the haematin compounds of the tissues are the enzymes catalase and peroxidase, in which haematin forms the prosthetic group or co-enzyme and is combined with specific proteins to form the enzyme. Another important haematin compound is the respiratory pigment cytochrome, shown by Keilin to exist in many aerobic plants and animal tissues. Cytochrome is a mixture of three haemochromogens. It is always accompanied by free haematin from which the haemochromogens are formed.

Cytochrome in the living cell acts apparently as a carrier of atmospheric oxygen between the indophenol oxidase of the cell and the many reducing bodies present. Indophenol oxidase, so called from its capacity to form indophenol from dimethyl-*p*-phenylene diamine hydrochloride and α-naphthol, appears, like several other oxidases, to be a copper-protein compound (p. 1). It would seem therefore that the close association of copper and iron, already found to exist in mammalian blood, extends also to most living aerobic tissues.

The non-haematin iron of the tissues may be present in both states of oxidation. Ferric iron appears to combine with non-diffusible phosphorus compounds, e.g. phosphatides and phosphoproteins, and probably also with other substances, but not with the simple proteins (Tompsett 1934). Ferrous iron apparently does not form such complexes ; if the iron in them becomes reduced it is liberated in ionisable form.

Tompsett (1935) gives figures for the ' inorganic ' (non-haematin) iron of tissues ranging from about 100 to 150 p.p.m. in the liver, spleen, vertebrae and ribs, down to roughly 5 or 10 p.p.m. in other organs.

AVAILABILITY OF IRON. As already pointed out, it is probable that only ferrous iron is capable of being absorbed from the intestine, and that ferric iron is not absorbed unless it is first reduced. It by no means follows, however, that all ferrous iron is available for absorption. It is generally held that haematin iron is hardly absorbed at all in either state of oxidation, and that the blood pigments have practically no dietetic value as suppliers of iron. On the other hand, Black and Powell (1942), administering a litre of blood by the mouth to one normal and two anaemic patients, found that 10 to 25 per cent of the iron was absorbed, of which only 5 to 10 per cent could be accounted for as being represented by non-haematin iron. They suggest that haematin may be broken down by bacterial action in the small intestine. Cooking, if sufficiently thorough, destroys the porphyrin complex and liberates available iron.

Besides porphyrins there are apparently other iron-containing organic compounds in which iron is not available, such compounds, for instance, as double phosphates of ferric iron with organic complexes in which the iron is not easily reducible, and which dissociate only slightly even in acid solution (cf. p. 252). The nature and amount of the phosphates present in food, and the calcium-phosphorus ratio, may thus have a considerable influence on the availability of iron. Pyrophosphates and metaphosphates in solution are well known to have the property of de-ionising ferric iron and interfering with its determination by colorimetric methods. Presumably they can also de-ionise it in the alimentary tract and prevent its reduction and absorption. Among the organic compounds of phosphoric acid which can combine with and de-ionise iron, is phytic acid (inositol hexaphosphoric acid). It is probably the phytic acid in wholemeal bread which makes this a less effective source of iron than white bread, although there is more than twice as much iron in wholemeal bread as in white bread (McCance and Widdowson 1942). It has been suggested that national flour, since it supplies 2 to 3 mg. more iron to the daily diet than white flour, is one of the factors responsible for the maintenance of a satisfactory nutrition level in recent years. High extraction flour, however, contains more phytic acid than does white flour : although the effect of the phytic acid may possibly be offset by a corresponding increase in phytase and also by fortification with calcium carbonate, it is still very doubtful whether national flour is better than white flour in respect of available iron.

The biological availability of iron in foods is ascertained primarily by experiments on animals previously made anaemic by bleeding or by special feeding. Chemically the two forms of iron compounds are distinguished by the fact that it is only available, ionisable or ‘ inorganic ’ iron which gives a red colour with $\alpha\alpha'$-dipyridyl on reduction with sodium hydrosulphite (Hill 1930). Instead of $\alpha\alpha'$-dipyridyl, Tompsett uses thiolacetic (thioglycollic) acid, which itself acts as a reducing agent. It is not known definitely whether iron which reacts with $\alpha\alpha'$-dipyridyl is always biologically available, or vice versa, but it is found in general that the content of available iron in foods as determined with $\alpha\alpha'$-dipyridyl or thioglycollic acid corresponds much more nearly than that of total iron with the results given by animal feeding experiments.

The availability of iron and its determination by chemical methods are further discussed on pages 252 and 261.

IRON REQUIREMENTS OF THE INDIVIDUAL: ABSORPTION AND EXCRETION. The daily requirement of an adult for iron is usually taken as being from 10 to 15 mg. Sherman's revised estimate (1937) is 12 mg.. He arrives at this figure by taking twenty-one recent experiments on iron metabolism by different workers and averaging their estimates of the daily iron intake required to keep the body in iron balance. The estimates range from 3·7 to 14·9 mg. and the average is 8 mg. to which Sherman adds 50 per cent as a ' factor of safety '. Of 8 mg. of food iron about 5 mg. will be in the form of available or ionisable iron. In a quantitative survey of the food actually consumed over a period of four weeks in 1937 in a household of two adults, a married couple, in London, on a weekly income of about £5, using McCance and Widdowson's figures for the average iron content of food, the daily intake of iron per head was estimated by me to be 12 mg., of which 7·5 were available. This is rather less than Widdowson and McCance's estimate (1936) of 16·8 mg. for a man and 11·4 for a woman, of which 10·8 and 7·9 mg. respectively were available. Their figures were the mean for 63 men and 63 women of middle class living on freely chosen diets. Daniels and Wright (1934), from experiments on iron retention in children from 3 to 6 years old, concluded that the optimum iron intake was from 8 to 14 mg. daily or 0·6 mg. per kilogram of body weight. The Committee on Food and Nutrition of the National Research Council of the United States suggest daily requirements increasing from 6 mg. for children under 1 year to 15 mg. for children of 13 to 20 years and 12 mg. for adults.

The mechanism of the absorption and excretion of iron is by no means clear. The urine contains hardly any, not much more than 0·01 mg. daily. The amount of iron in the faeces follows closely that in the food, and we are met here by the same difficulty as with copper, lead, etc., of distinguishing between iron which is absorbed into the body and excreted into the intestine and iron which passes through without being absorbed at all. Since the bile and other secretions finding their way into the intestine contain the merest traces of iron, any excretion which takes place must be through the intestinal mucous membrane. McCance and Widdowson (1937, 1938) give reasons for thinking that the absorption and excretion of iron in the alimentary tract are much less than they were formerly thought to be. They find that even when the normal iron content of the body has been more than doubled by heavy medicinal doses, the body seems to have little or no capacity for excreting it. The excess of iron seems to be all stored and does not increase the concentration in the blood plasma. They consider that the diffusion of iron into and out of the epithelial cells of the intestinal wall is controlled by the relative concentrations of free inorganic (presumably ferrous) iron in the plasma and in the contents of the intestine. Normally there will always be a small amount of iron in the intestine from food, but the amount of ionisable inorganic iron will be low. The diffusion gradient across the cell walls can never be a steep one, except when large quantities of iron are given medicinally, so that normally both absorption and excretion of iron are extremely small. With the iron naturally present in food there is a very small positive iron balance, a milligram or so daily, whereas a negative balance is never found except at extraordinarily low intakes of iron. Practically the whole of the iron found in the faeces is derived from food iron which has passed through the alimentary tract without being absorbed.

McCance and Widdowson's hypothesis would seem at first sight to be opposed to the accepted view that the body should have 10 to 15 mg. of iron daily in food. If there is practically no excretion of iron it must follow that the bodily store is constantly being turned over and re-used, and that a very small intake of iron should be all that is required to replace loss and to maintain equilibrium. Iron is, however, absorbed from food with such extreme difficulty that a relatively large intake is necessary to ensure the effective absorption of even a minute quantity. In this connection women are in a different position from men, since they are subject to periodic losses of iron, 10 to 30 mg. each month, and require therefore a larger daily intake than men. An intake of 15 mg. per day in food may be barely sufficient for women, while unnecessarily high for men. Orr (1934) suggested that the optimum requirement for a woman may be 20 mg. daily and for a man less than half of this. In this connection, however, Leverton's results may be noted. In an experiment on four young adult women she found them in iron balance with not more than 4·5 to 6·5 mg. daily. In a man, iron balance has been maintained for a short time on as little as 1 mg. daily (Lintzel 1929), indicating an extremely low excretion of iron.

Hahn and others (1943) have used a radioactive isotope of iron to investigate this problem. They find, in agreement with McCance and Widdowson, that normally the absorption of iron from food is very small indeed. If, however, a person becomes anaemic from any cause, absorption of iron from food may be as much as five to fifteen times the normal. They take the view that the passage of iron through the intestinal wall is hardly possible so long as the plasma is ' physiologically saturated ' with it, but if the concentration in the plasma falls below this point iron is more easily absorbed from food. Widdowson and McCance (1944) point out, however, that even with the aid of radioactive isotopes it is difficult to measure the real absorption of an element from food.

In children and adolescents who are growing fast the absorption of dietary iron is very much higher than in adults (Josephs 1939, Porter 1941).

It has been suggested that since the family budgets of the working classes seldom provide more than 8 to 9 mg. of iron daily, more than half of the population is living in a state of chronic iron starvation. It is an undoubted fact that many children and nursing mothers in the lowest income groups have a deficiency of haemoglobin in their blood. In these groups the intake of iron is low, but the total food intake is also much below the optimum. It would seem from the figures given on page 251 for iron in foods that iron deficiency is not so much a result of cheap food (potatoes, cereals and pulses) as of insufficient food, and that iron intake on the whole varies directly with the amount of food consumed. It is true that the food of the lower income groups is, in the aggregate, slightly poorer in iron than that of the higher income groups, owing largely to the relative proportions of meat, and this fact has prompted the suggestion that certain foods should be fortified with iron as has already been done with calcium and vitamins. There are, however, several factors other than mere intake of iron which influence its eventual utilisation in haemoglobin formation. Firstly, its state of combination in the food and the effect of phosphates and other food constituents on its reducibility to the ferrous condition in the alimentary tract. This has already been discussed on page 241. A second factor influencing the absorption of iron is ascorbic acid. Iron administered medicinally is not readily assimilated if ascorbic acid is withheld. A further factor is the intake of animal protein. It is probable that lysine (a diamino-caproic acid), which is supplied in greater quantity by animal than by vegetable protein, is one of the amino-acids essential for haemoglobin synthesis, and that a sufficient intake of animal protein is necessary for full utilisation of dietary iron (cf. *M.R.C. Special Rept. No.* 252, 1945).

It is clear that before fortification of particular foods with iron is undertaken full consideration should be given to the conditions influencing its absorption. It is quite possible that the actual amount of iron in food is of less importance than the various factors which promote or inhibit its absorption and utilisation (Leverton and Marsh 1942). Obviously extra iron is often of value, if given in sufficient quantity, as evidenced by the success attending the administration of iron tonics, but the chief cause of nutritional anaemia and low haemoglobin index is inadequate nourishment, and the first requirement is not more iron but more food.

Since the amounts of iron absorbed and excreted are very small compared with the total amount contained in food, the assessment of the state of iron balance in any individual will depend upon the measurement of very small differences in relatively large quantities of iron, and will call for extreme care and accuracy in both sampling and analysis. The exact determination of small amounts of iron in organic material is by no means easy, and the problem of iron absorption and excretion may be largely one of accurate chemical analysis.

ACTION OF IRON ON FATS. Iron promotes the oxidation of fats and is liable to cause a tallowy flavour in milk and butter. There is a great difference between ferrous and ferric iron in the effect on milk. As little as 0·7 p.p.m. of ferrous iron causes an off-flavour in half an hour, but to produce the same effect with ferric iron requires 60 p.p.m. acting for 24 hours. Ferrous iron therefore appears to have a catalytic effect equal to that of copper in promoting oxidation of fats. Ferric iron is known to form complexes with the proteins of milk, and in this form it is relatively inactive in causing off-flavour. The normal iron content of milk is very small, about 0·5 p.p.m.

One or two parts of ferric or ferrous iron per million of butter will cause a tallowy taste in a short time, and it is stated that 2·5 p.p.m. can be recognised by a distinct metallic taste. In New Zealand it is considered that butter should not contain more than 1·5 p.p.m. of iron or 0·2 p.p.m. of copper.

Minute traces of iron promote the oxidation of olive oil. Even 1 p.p.m. of ferric oleate has a pronounced effect. Different oils vary in their sensitivity to iron (Garner 1936).

IRON AND PLANT LIFE. Small amounts of iron are essential to plant growth, if only for the production of the respiratory pigment cytochrome. It is probably also concerned in the synthesis of chlorophyll. Lack of iron causes chlorosis in plants, particularly in apple trees, and this is very liable to occur on calcareous soils poor in humus, iron in such soils being largely in an insoluble unavailable form. The small amount of iron necessary to plants is made available by the action of humus, which acts as an iron carrier and prevents it from being all precipitated as basic carbonate in alkaline soils (Clark and Sieling 1936). The application of soluble salts of iron to a calcareous soil reduces chlorosis, but on the other hand excess of soluble iron is toxic.

Glauconitic greensand has been found to be toxic to plants unless lime is present, owing to the iron of the glauconite being soluble in slightly acid media (Kelly 1923).

The behaviour of iron in soils appears to be similar to that of aluminium. The more acid the soil the more liable is it to contain soluble salts of iron. Plants which grow well on acid soils tolerate iron much more readily than plants growing on calcareous soils. Iron, however, differs from aluminium in that small amounts are essential to plants, whereas there is no evidence that aluminium is essential. According to Maquenne and Demoussy (1920) ferrous salts are much more toxic to young plants than ferric salts. It has been suggested that the manganese of soils acts as an oxidising agent and regulates the relative proportions of ferrous and ferric iron (p. 300).

Iron, in common with several other metals, appears to be necessary for the growth of the mould *Aspergillus niger*. It is also closely associated with the so-called ‘ iron bacteria ’. These organisms have the power of taking up iron either from water or from the surfaces of iron pipes and tanks. They can abstract iron from water containing as little as 0·1 p.p.m., and some of them can take up manganese with equal facility. Under favourable conditions they may multiply in water to an enormous extent and produce reddish-coloured slimy deposits containing ferric hydroxide, which may stop up pipes and give the water an offensive smell. A slightly acid water containing organic matter is the most suitable for the growth of these bacteria. If the growth becomes serious it is necessary to neutralise any acidity in the water, and to remove organic matter, iron and manganese as far as possible. This may be done by treatment with lime followed by sedimentation and filtration.

IRON HAZE OF WINES. Wines containing more than about 8 p.p.m. of iron are apt to become cloudy owing to the precipitation of organic complexes of ferric phosphate. The turbidity becomes objectionable with an iron content of more than 15 p.p.m. South African grapes contain only 1 to 3 p.p.m. of iron, but the wine often takes up iron from various sources during manufacture and may contain from 7 to 25 p.p.m. (de Waal 1932). Certain French wines contain from 14 to 40 p.p.m. of iron (Hugues 1929) and German wines 2 to 20 p.p.m. and occasionally much more. Special care must therefore be taken to prevent the occurrence of cloudiness. One of the causes of excessive iron in wine is treatment of the must with sulphur dioxide or sulphites followed by contact with iron. If fermentation is carried out in iron-free vessels most of the soluble iron disappears from the wine (Byrne and others 1937).

Iron haze occurs only under conditions which favour oxidation. Ferrous iron does not cause cloudiness ; it can exist in wine in an inorganic form and does not readily combine with organic phosphates, whereas ferric iron tends to form insoluble complexes. The ordinary clarifying agents will remove ferric iron completely from wine but not ferrous iron, which remains in solution and may afterwards give rise to cloudiness on oxidation. In presence of free sulphurous acid iron remains in the ferrous condition and turbidity does not occur. Sometimes when a wine shows a slight haze due to ferric phosphate it has the curious property of clearing when exposed to light in white glass bottles, owing to reduction of ferric phosphate to the more soluble ferrous phosphate, and of becoming hazy again when kept in the dark. Wines containing much alcohol and little acid are the most prone to iron haze. According to Moreau and Vinet (1917) it may be prevented by adding 0·5 to 1 p.p.m. of citric acid to the wine, the action being perhaps similar to that of citrates in preventing the precipitation of iron phosphate in analytical work.

Iron, whether ferric or ferrous, can be removed from wine by precipitation with potassium ferrocyanide, a process introduced by Moslinger about 1918 and for some time kept secret. Traces of copper and zinc, which occasionally cause persistent cloudiness, are also removed by this treatment. In order to avoid the addition of too much ferrocyanide the iron content must first be determined. Potassium ferrocyanide is then added in amount necessary to precipitate nearly all the iron, leaving from 2 to 4 p.p.m. in solution. This process has been sanctioned in Germany provided that no ferrocyanide remains in the wine. Unless it is carried out under skilled supervision there is a danger of the wine becoming contaminated with hydrogen cyanide by gradual decomposition of residual ferrocyanide. Monnier (1920) found a bright blue sediment of ferric ferrocyanide in some white wines which had evidently been clarified by this process. Malvesin in 1921 reported an instance of an Algerian wine containing an excess of ferrocyanide which was mixed at Rouen with a wine containing iron, with the result that the mixture threw down a considerable precipitate of Prussian blue.

Apart from its use as a specific precipitant for iron in wine, potassium ferrocyanide was used at one time in conjunction with zinc salts as a general fining agent, the voluminous zinc ferrocyanide precipitate carrying down with it all suspended matter. About 1 lb. of potassium ferrocyanide and 1 lb. 2 oz. of zinc sulphate were used for 1,000 gallons of wine. This process was forbidden in France in 1907 and in Germany in 1909. Potassium ferrocyanide has also been used in this country for the clarification of vinegar (Hamill 1908). It was added in the proportion of about 0·01 per cent and precipitated the proteins of the vinegar, thus carrying down suspended matter. Vinegar so clarified may form a deposit of Prussian blue and in extreme instances pickles made with it may become blue.

Tests for residual ferrocyanide in wine depending on the formation of Prussian blue are given by Reichard (1927). Hydrogen cyanide may be determined in presence of excess of ferrocyanide by Feld's method—boiling with magnesium chloride in neutral solution.

IRON IN FOODS

Natural Iron. A rough classification of foods according to iron content is as follows. The data have been taken in part from McCance and Widdowson's tables (1940).

Nil to 10 p.p.m.—white bread and flour, pearl barley, rice, milk, butter, cheese, most sea-fish, most fresh fruits, most green vegetables, potatoes, milk powder.

10 to 50 p.p.m.—shredded wheat, wholemeal flour, oatmeal, cornflour, arrowroot, beef, mutton, veal, chicken, bacon, herrings, sprats, nuts, dried fruit, spinach, cress, radishes, peas, coffee, chocolate.

50 to 100 p.p.m.—bran, egg yolk, some kinds of poultry and game, whelks, mussels, oysters, lentils, haricot beans, mustard, pepper, black treacle.

Above 100 p.p.m.—liver, kidneys, cockles, winkles, tea leaves, parsley, cocoa, ginger, curry powder (750 p.p.m.).

The large amount recorded for curry powder can hardly be natural, and it is possible that yellow ochre may have been used for colouring.

The iron contents of different grades of flour are roughly as follows :

White flour (70 per cent extraction)	9 p.p.m.
80 per cent flour	16 ,,
85 per cent flour	21 ,,
Wholemeal flour	30 ,,

Cows' milk contains only 0·1 to 0·65 p.p.m. of iron with a mean of 0·3 p.p.m. (Johnston 1944), and the amount cannot be influenced by feeding. The iron requirements of the calf in the first few weeks of life are mainly met from that stored in the liver during foetal life.

Stiebeling (1932) groups vegetables and fruits as follows according to their iron value in nutrition.

Poor (0 to 4 p.p.m.)—most fruits and fruit juices.

Fair (4 to 8 p.p.m.)—fruits, seed pods, blanched leaves and stalks, roots and herbs.

Good (8 to 16 p.p.m.)—potatoes, thick green stalks and leaves.

Excellent (over 16 p.p.m.)—mostly leguminous plants and green leaves.

Vegetables may lose up to 20 per cent of their iron when boiled in water (Sheets and others 1931) and broccoli as much as 50 per cent (Flanley and Johnson 1932).

For the purpose of assessing nutritive value a knowledge of total iron is of less value than that of available iron. Vegetables and fruit, although generally low in iron, contain most of it in available form : in most foods which are high in iron a smaller proportion is available. In beef and mutton only 10 to 25 per cent of the iron is available, but the proportion is greatly increased by cooking (Oldham 1941). Green-leaf vegetables contain 60 to 70 per cent of their iron in available (inorganic) form and potatoes and cereals 90 to 100 per cent. In eggs practically the whole of the iron is available and also in calf liver, where it probably exists mainly in the form of ferritin (p. 240) and not haematin.

The figures for available iron in fruits and vegetables are sometimes unaccountably low. Thus in mulberries, strawberries, plums, walnuts, mustard and cress only half of the total iron is available, which indicates that there must be several other organic compounds of iron, besides the iron porphyrins, which do not react with αα′-dipyridyl on reduction. Hill (1930) in fact considered that in many foods haematin compounds account for only a small fraction of the iron that is resistant to reaction with dipyridyl and a reducing agent.

Tables showing the total and available iron in about 300 different foods are given in a report of the Imperial Bureau of Animal Nutrition (1938).

Adventitious Iron. Iron is often introduced into beverages which are then claimed to possess special dietetic or tonic qualities. Schönfeld (1914) records that in Germany beer has been produced with iron contents ranging from 2 to 140 p.p.m. without making it unpalatable. The chief difficulty is to prevent the development of cloudiness owing to precipitation of ferric phosphate, tannate, etc. Heavy dark beer containing much sugar and a minimum of phosphoric acid and tannins will hold iron with the least effect on flavour or danger of precipitation. Light beers on the other hand are easily affected in appearance, colour and taste by as little as 1 p.p.m. of iron (Gray and Stone 1938). Iron yeast-presses may cause astringency and iron haze in beer (Matthews 1929). 1 p.p.m. of iron gives a distinct taste to drinking-water.

In canned foods considerable quantities of iron may gain access to the food if the tin coating becomes corroded. This is especially liable to occur in lacquered cans containing fruit. The lacquer may give way in places where it has been strained in forming the can, and thus a small area of metal is exposed to the action of the fruit acids. The tin is first dissolved and the acids then attack the iron with evolution of hydrogen and consequent ‘ blowing ’ of the can. Such cans are known as ‘ hydrogen swells ’ (p. 143). The contents contain iron in amount roughly equivalent to that of the hydrogen formed. In one instance canned loganberries which showed hydrogen swelling contained from 150 to 320 p.p.m. of iron (Monier-Williams 1926). The so-called tin taste in some canned foods is due to iron.

A further point to which attention has been drawn is that the iron intake in food may be greatly increased by iron derived from cooking vessels such as chipped enamel saucepans (Klumpp 1934, Widdowson and McCance 1943).

Iron may cause discoloration of foods either by precipitation as sulphide or by combination with tannic acid. Canned lobster, crab and shrimps, and also canned sweet corn, are liable to become blackened by iron owing to their high sulphur content and production of hydrogen sulphide by bacterial action. It has been suggested that discoloration of foods by iron sulphide may be prevented by coating the interior of the container with wax or lacquer containing zinc oxide, colourless zinc sulphide being formed in preference to iron sulphide. Zinc salts have at times been added to canned foods with this object in view. It may be that the relative freedom of canned corn from discoloration in former days was due to traces of zinc chloride used as a flux in soldering the cans. According to Fellers and Harris (1939) the copper of haemocyanin (p. 4) may sometimes be concerned in the sulphide blackening of canned crab.

In one instance calves-foot jelly in a glass jar closed with a tin-plate cap and vulcanised rubber ring was found to be badly blackened by iron sulphide. The rubber appeared to have broken down and corroded the metal cap, and iron had diffused into the jelly where it had become converted into black iron sulphide by hydrogen sulphide presumably liberated from the rubber.

Discoloration of food by ferric tannate can take place only in presence of air. It is sometimes seen in canned strawberries, which may be darkened by as little as 2 to 5 p.p.m. of iron. Raspberries are discoloured by 10 p.p.m. of iron and turned to a dark purplish brown by 40 p.p.m. (Morris and Bryan 1936). Golden syrup in cans is liable to become blackened after long storage, owing to combination of iron with hydroxy-acids. These hydroxy-acids are formed by the action of iron and oxygen on reducing sugars in presence of traces of acid (de Whalley 1944). It is well known that tea or coffee, if made in an iron pot, may give a greyish-black infusion. A similar colour is seen when highly ferruginous water is used for making tea or diluting spirits, owing to the formation of ferric tannates. In one instance whisky kept in a wooden cask was found to have become blackish in colour from an iron nail accidentally dropped into the cask. Iron may be partly responsible for the darkening of potatoes during cooking, and this discoloration is accentuated if iron gains access from cooking vessels.

Iron oxide, known as Armenian bole, is sometimes added as a colouring matter to such foods as potted meats, sausages, anchovy and bloater pastes, and confectionery. The original Armenian bole seems to have been a red ferruginous earth, but the substance now used is made in this country by calcining ferrous sulphate (copperas). The product of the calcination is treated with water, the suspended particles of iron oxide allowed to settle and the sediment dried and powdered. The product is used for paint manufacture, polishing powder, rouge, etc., as well as for colouring food. Usually no attempt is made to purify it for use in food, and it is often seriously contaminated with arsenic. A sample sold in 1926 for this purpose was found to contain as much as 800 p.p.m. of arsenic and over 6 per cent of zinc oxide (*Analyst*, 1926, **51**, 412). Taking 0·5 to 1·0 per cent as the amount of Armenian bole added to some foods, the above material would contribute from 4 to 8 parts of arsenic per million parts of the food. If ferric oxide is used to colour foods it should be prepared from recrystallised ferrous sulphate.

On the further question whether iron oxide, even when pure and free from arsenic, should be used at all for colouring foods, it must be admitted that on health grounds it is difficult to advance any strong objection to its presence in small quantities. Calcined ferric oxide is soluble with difficulty in acids and may be considered inert in food, but when added in such large amounts as 10 per cent, as recorded for anchovy sauce in the report of the Arsenic Commission (1903), it can hardly fail to interfere with digestion. Nowadays such excessive quantities are seldom met with ; 0·5 to 1 per cent seems to be the more usual proportion. A somewhat similar admixture of inert mineral matter with food is to be found in the facing of rice with talc, for which a limit of 0·5 per cent is usually accepted, and the same limit might be applied to ferric oxide. It may be noted, however, that the Royal Commission of 1903 considered that mineral pigments should not be used at all in food.

Particles of metallic iron (' iron filings ') and iron oxide are occasionally found in tea ; 1·4 per cent in one sample examined in 1926 (*Analyst*, **51**, 573) and as much as 8 per cent in a sample examined in 1942 (*Brit. Food J.*, **44**, 68). Its presence is alleged to be due to the practice of blending ' sweepings ' with the lower grades, although why sweepings should contain so high a proportion of iron filings is not explained. Hassall in 1876 referred at length to the presence of iron in ' lie ' tea, and showed that it was not iron filings but magnetic oxide of iron. Lie tea is a very low grade article made up from tea dust and mineral matter. According to Hassall it has often been found to contain so much iron oxide that this must have been added intentionally, possibly with the object of obtaining a darker infusion due to iron tannate, or perhaps merely as a make-weight since flakes of magnetic oxide are somewhat similar to tea in appearance. The instances recently recorded indicate the survival of a practice long regarded as obsolete, in spite of the close control over tea exercised by the Customs and Excise Department. Iron, if dissolved to any appreciable extent, would of course be highly detrimental to any but the very lowest grades of tea owing to the blackening of the infusion. Particles of iron oxide would tend to become segregated in transit and to accumulate in the lower layers of tea, and this may be the chief reason for its occasional presence in such large amounts.

The facing of tea with a mixture of turmeric, Prussian blue and gypsum or china clay, another practice thought to be obsolete, is still done on a very small scale, usually with the so-called ' gunpowder ' tea (*Ann. Rept. C.M.O.* 1936). True gunpowder tea is a high grade of green (unfermented) tea in which the leaves are rolled up so as to present a granular appearance. The name is also given to a lower grade article containing tea dust and binding materials and faced to improve its colour. Gunpowder tea is used for blending or flavouring and is rarely drunk in the unblended state. Comparatively little is sold for consumption in this country, the greater part being exported, in normal times, chiefly to Morocco. It is, or used to be, the practice of the Customs and Excise Department to admit faced tea provided that it does not contain more than 2 per cent of facing matter (*Analyst*, 1899, **24**, 333). Faced tea is apt to be contaminated with arsenic from the use of impure Prussian blue, as much as 0·3 gr. per lb., or even more, having been found in comparatively recent years. The facing of tea is prohibited in the United States and in several other countries.

THE DETERMINATION OF IRON IN FOOD

DESTRUCTION OF ORGANIC MATTER. The chief point to be noted in the destruction of organic matter before the determination of iron is the possibility of loss of iron by volatilisation as chloride. Ferric chloride is volatile at temperatures as low as 450°. In the dry ashing of materials containing salt or chlorine, calcium carbonate or other alkali should be added to prevent loss of iron.

Woiwod (1947) finds that, with foods high in phosphorus, low results for iron may be due to the formation, in the ash, of iron complexes, probably metaphosphates, which resist acid hydrolysis. He takes the ash to dryness with a small quantity of hydrochloric acid, and treats the residue with ammonium sulphide (cf. Jackson 1938). The resulting ferrous sulphide, without separation from the phosphates present, is dissolved in hydrochloric acid, and the iron determined colorimetrically with $\alpha\alpha'$-dipyridyl (p. 261). Davidson (1931) points out that, with ash rich in silica, extraction with dilute hydrochloric acid gives low results for iron owing to retention by silica, and that silica must first be volatilised by heating with hydrofluoric acid. Oxidation with sulphuric acid in conjunction with nitric acid or hydrogen peroxide entails no risk of loss of iron. The Association of Official Agricultural Chemists recommend wet oxidation with perchloric and nitric acids (*J.A.O.A.C.*, 1941, **24**, 215).

COLORIMETRIC DETERMINATION WITH THIOCYANATE. This well-known method is often used for the determination of iron in small quantities such as are contained in foods and biological material. It depends upon the formation of red ferric thiocyanate in acid solution. According to Bent and French (1941) the actual coloured substance is the ion $FeCNS^{++}$. Any ferrous iron present must first be oxidised to ferric iron. When the iron content of the material is high and there is practically no phosphate present the ash, dissolved in dilute nitric acid, may be treated with excess of ammonium or potassium thiocyanate and the solution compared direct with standards. With foods high in phosphates and low in iron the determination is not so simple, and the following points must be born in mind.

Interference by Phosphates. Pyrophosphates and metaphosphates interfere greatly with the development of the red colour with thiocyanate, owing to partial de-ionisation of iron. Orthophosphates also interfere but not to the same extent. According to Leeper (1930) as little as 0·08 mg. of P_2O_5 as metaphosphate, or 0·40 mg. as pyrophosphate, in 50 ml. of solution will prevent the full production of colour; with orthophosphate 100 to 200 mg. must be present before the red solution begins to show a yellowish tint. The ash of foods containing phosphorus always contains pyrophosphates, and for this reason it is better to use a wet oxidation method or else to boil the solution of the ash for some time with acid, in order to convert meta- and pyrophosphates into orthophosphate (cf. Woiwod, p. 257). Even in wet oxidation pyrophosphate may be formed if too little sulphuric acid is present (Tompsett 1934). For foods very high in phosphate, e.g. milk, Marriott and Wolf's acetone method (1906) may be used. The solution is diluted with an equal volume of acetone before adding potassium thiocyanate, and this reduces greatly the likelihood of interference by phosphates. The presence of acetone increases the sensitiveness of the reaction and the stability of the colour (Woods and Mellon 1941). In the method recommended for iron in beer by the Association of Official Agricultural Chemists (*J.A.O.A.C.*, 1941, **24**, 215) the thiocyanate colour is developed in an acid medium containing about 75 per cent of methoxyethanol (methyl cellosolve), $CH_3O.CH_2.CH_2OH$. A high concentration of thiocyanate (4 per cent of KCNS in the solution as diluted for colorimetry) also helps to lessen interference by phosphates.

Elvehjem and Hart (1926) remove phosphoric acid by boiling the ash with alkali to convert pyrophosphate to orthophosphate, and precipitation of the latter with ammonium molybdate. On adding potassium hydroxide to the filtrate, iron is precipitated as hydroxide and the excess of molybdate remains in solution. The iron hydroxide is then dissolved in hydrochloric acid and determined colorimetrically with thiocyanate (cf. Woiwod, p. 257, and Howe, p. 262).

According to Woods and Mellon (1941) fluorides and oxalates also interfere with the reaction by forming colourless complexes with iron (cf. p. 372).

Extraction of Colour with Organic Solvents. With small amounts of iron the delicacy of the method is greatly increased by extracting the ferric thiocyanate with organic solvents and carrying out the colour comparison on the extract. Ether has been used for the extraction but amyl alcohol is preferable. Stokes and Cain (1907) use a mixture of 5 parts of amyl alcohol and 2 parts of ether, and Stugart (1931) *iso*-amyl alcohol (cf. p. 49). Thompson (1944) uses *iso*-butyl alcohol. A further advantage of extraction with these solvents is that it prevents interference by phosphates with foods high in phosphorus (Farrar 1935). Extraction of ferric iron compounds from acid solution with organic solvents is a principle of fairly wide application in the separation of iron from other metals (cf. Ramsay 1944).

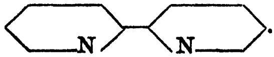
Acidity of Solution and Stabilisation of Colour. Unless the solution is kept strongly acid, ferric thiocyanate, or the complex ion responsible for the colour, tends to dissociate and the full colour is not developed. In this state of partial dissociation the depth of tint is very sensitive to slight variations in acidity and concentration. According to McFarlane (1932) the best degree of acidity, corresponding to the largest proportion of undissociated molecules of ferric thiocyanate (or of $FeCNS^{++}$ ions), is about 2N hydrochloric acid. Dissociation is also minimised by using a large excess of potassium thiocyanate. With increasing concentration of thiocyanate the colour becomes deeper until about 4 per cent of potassium thiocyanate is reached, after which it remains constant. If too much thiocyanate is added the colour becomes slightly brownish red.

Ferric thiocyanate is easily reduced in aqueous solution with consequent loss of colour, and for full colour development it is necessary always to have an oxidising agent present. Potassium persulphate and hydrogen peroxide have been used to keep the iron in the ferric state, but it seems preferable to carry out the determination in dilute nitric acid solution. Walker (1925) uses 2 per cent of nitric acid and in Smith and Cooke's method (1926) as much as 10 per cent is present. With nitric acid a more intense coloration is obtained than with hydrochloric or sulphuric acids. For the same reason other metals besides iron capable of existing in more than one state of oxidation may affect the colour development unless they are kept fully oxidised. Nitric acid should not be used to extract iron from the ash of foods but should be added at a later stage (p. 266).

According to Walker nitrous acid may give a red colour with thiocyanate and should therefore be removed. On the other hand, nitric acid if free from nitrous acid does not readily oxidise ferrous iron. He therefore uses nitric acid containing nitrous acid to oxidise iron, and then adds hydrogen peroxide to convert the remaining nitrous acid to nitric acid before proceeding with the colorimetric thiocyanate determination.

Silver, copper, nickel and cobalt, if present in relatively large amounts, are stated to interfere with the thiocyanate method, and if more than 6 p.p.m. of zinc are present the colour may be bleached (de Brouckère and Gillet 1933). Smirk (1927) compares the ferric thiocyanate colour with standards prepared from cochineal and methyl red in varying proportions. Dovey's device (1918) is also worth noting. After addition of potassium thiocyanate he places two-thirds of the solution in one Nessler cylinder and one-third in another. He then adds standard ferric iron solution to the cylinder containing the one-third until its tint is the same as that of the other two-thirds. The liquids being similar in composition any differences due to variations in tint are eliminated.

Control tests : Contamination with Iron. When dealing with small amounts of iron it is a matter of some difficulty to secure the requisite freedom from iron of reagents, apparatus, etc. Potassium thiocyanate itself always contains traces of iron, and nitric acid if kept for a long time in glass may take up iron from it. Smith and Cooke (1926) give methods for obtaining iron-free reagents. Elvehjem and Hart's method (1926) of hydrolysing pyrophosphates by boiling with alkalies has been adversely criticised as giving high results owing to extraction of iron from the glass by alkali. Cork and rubber stoppers are liable to contribute traces of iron, and care must be taken at all stages of the work to exclude dust. It is obvious that no knives or other articles of iron or steel must come into contact with the material under examination. Blank experiments must, of course, always be carried out on the reagents and apparatus, but unless every care is taken to reduce the iron of the blank to the lowest possible amount the delicacy of the determination will suffer.

DETERMINATION OF AVAILABLE IRON WITH αα′-DIPYRIDYL. This reagent was introduced by Hill in 1930. It is a crystalline substance of structural formula . Unfortunately it is difficult to prepare and very expensive. It gives a deep red colour with ferrous iron, but does not react with ferric iron unless this is previously reduced. The chief use of αα′-dipyridyl is to differentiate between haematin (porphyrin) iron, which does not react with it in either state of oxidation, and the so-called ionisable, available or inorganic iron, which reacts with dipyridyl either directly, if in the ferrous state, or on reduction. As already pointed out it is probable that several organic complexes of iron occurring in foods are either reduced with difficulty or behave like haematin towards dipyridyl, and in fact in some foods haematin iron seems to form only a small fraction of the non-reacting iron.

Ferric iron in food is practically always in the form of non-diffusible complexes with phosphoproteins, etc. (Tompsett 1934). The term ‘ inorganic ’ iron does not necessarily imply that the iron is not in organic combination, but that it can be converted by reduction into a dialysable form, whereas haematin iron is so closely bound in the porphyrin ring that it cannot be obtained in a dialysable form unless the ring is broken.

Since heat may cause partial decomposition of haematin and other organic iron compounds it is obvious that in nutrition investigations the analysis should be carried out on the cooked food as eaten, and it is probably inadvisable to subject the samples to oven drying or any further heating before analysis.

If organic matter is destroyed by ashing or wet oxidation, $\alpha\alpha'$-dipyridyl can be used with a reducing agent to determine total iron. It has been claimed to have the advantage over thiocyanate that since iron is determined in the ferrous state pyrophosphates do not interfere. Howe (1944), on the other hand, finds that the presence of pyrophosphate leads to low results, and that the ash must be boiled for at least 15 minutes with diluted hydrochloric acid (1 : 1) to convert pyro- into orthophosphate (cf. Woiwod, p. 257).

Shackleton and McCance (1936) give details of the determination of ionisable iron in foods by the dipyridyl method using hydrosulphite as the reducing agent. The authors state that it is possible, with a comparator, to determine the iron content of the solution (20 ml.) to the nearest 0·0025 mg. The method is thus quite as sensitive as that with thiocyanate and the colour is permanent. Possible errors due to incomplete extraction of iron are reduced to a minimum if the sample is carefully ground or pulped in a mortar and extraction with buffered hydrosulphite solution allowed to proceed overnight. A certain amount of contamination of the dipyridyl red with yellow pigments usually makes direct colorimetry impossible, hence the use of a comparator. A further possible source of error is the adsorption of the coloured complex on to insoluble matter. The addition of 30 per cent of alcohol to the solution prevents this adsorption with flesh foods but not with vegetables and fruits : with these it is necessary to filter the solution before adding dipyridyl. As with the thiocyanate determination great care must be taken to avoid contamination of the apparatus and reagents with iron.

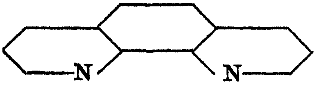
The figures found by various investigators for ionisable iron in foods (McCance 1939) indicate that the results depend in great measure upon the way in which the determination is carried out. Scharrer (1934) favours a solution of sodium sulphite just not alkaline enough to precipitate alumina. Some analysts use an ammoniacal solution with hydroquinone as the reducing agent while McCance (1939) works with an acetic acid-acetate buffer at pH 5·5. To this extent, therefore, the method is empirical, and much remains to be done before the results can be closely correlated with those obtained by animal experiment. Perhaps the availability of iron is itself a variable factor depending upon the reduction potential existing at any moment in the gut, and a close correlation cannot be expected.

Cooper (1935) has found that 2:2′:2″-tripyridyl is the most sensitive reagent for iron in sea-water. It will detect 1 part in 1,000 million. According to him the iron in sea-water amounts to less than 2 parts per 1,000 million (2 mg. per cubic metre) and is all in the ferrous state. Goldschmidt's estimate (1937) is 60 parts per 1,000 million.

DETERMINATION WITH THIOLACETIC (THIOGLYCOLLIC) ACID. Tompsett (1934) has made considerable use of thiolacetic (thioglycollic) acid, $CH_2(SH)CO_2H$, for determining inorganic iron. This method was introduced by Lyons in 1927. Thiolacetic acid gives a purple colour with ferrous iron in ammoniacal solution and differs from αα′-dipyridyl in being itself a reducing agent so that no addition of hydrosulphite is necessary. When using thiolacetic acid Tompsett removes protein with trichloracetic acid (cf. also Moir and Andrews 1940).

Thiolacetic acid was used by Shackleton and McCance (1936) in preference to dipyridyl for determining total iron in the ash of foods. The presence of pyrophosphate does not interfere with the development of the colour, but sodium silicate has a bleaching action (Strafford 1933). Other heavy metals do not interfere unless present in relatively large amounts (0·1 per cent), when they may affect the delicacy of the method. The addition of sodium citrate often prevents interference by other metals. It should be noted that in acid solution nitrites give a red colour with thiolacetic acid (Clark 1936).

DETERMINATION WITH *o*-PHENANTHROLINE. Saywell and Cunningham (1937) use *o*-phenanthroline



as a colorimetric reagent (cf. structural formula of αα′-dipyridyl, p. 261). The compounds of this body with iron were originally investigated by Blau in 1898. It gives an intense red colour with ferrous iron in ammoniacal solution due to a complex ion, $[Fe(C_{12}H_8N_2)_3]^{++}$, which changes to blue on oxidation. Organic matter is first destroyed by wet oxidation with sulphuric and perchloric acids, and iron is reduced with hydroxylamine. An alcoholic solution of *o*-phenanthroline is added, the solution made slightly ammoniacal and the colour matched with standards.

According to Vahlquist (1939) the colour development in ammoniacal solution, although rapid at first, is either incomplete or only slowly attains its maximum. The best conditions are found at pH 1·65 to 2·85. If the solution is more acid development of colour is slower. Bandemer and Schaible (1944) use hydroquinone as a reducing agent, and adjust the pH of the iron solution to 3·5 by addition of sodium citrate. For accurate results the hydroquinone and *o*-phenanthroline must be added before the sodium citrate.

Pringle (1946) has improved the method and applied it to the determination of iron in cereals.

TITRATION WITH TITANOUS CHLORIDE. Knecht and Hibbert's method as modified by Klumpp (1934) has been recommended by a Medical Research Council Subcommittee on Analytical Methods (1943) as being the most accurate for the determination of iron in blood and blood pigments. It is carried out on 1 to 5 ml. of blood, ashed at a low temperature. The ash is dissolved in a few millilitres of diluted sulphuric acid and the solution titrated with an approximately 0·2 per cent titanium chloride solution previously standardised against an iron solution of known strength (1 ml. = 1 mg. Fe). The titration is carried out in an atmosphere of carbon dioxide, and potassium thiocyanate is added as an indicator. A diagram of the arrangement of the titration apparatus is given in Klumpp's paper. The method as described is applicable to quantities of iron of 0·5 mg. and over, and Ramsay (1944) has modified it for the determination of 10 μg. of iron in micro-analytical work. McFarlane (1932) employs a potentiometric titration with titanous sulphate for quantities of iron not less than 0·15 mg. Copper interferes with the titanium chloride titration and must be removed. Pyrophosphates also, if present in amount large enough to cause decolorisation of the thiocyanate indicator, must be removed (p. 258).

TITRATION WITH PERMANGANATE. The ordinary determination of iron by reduction followed by titration with permanganate is suitable for relatively large amounts of iron, from 1 to 10 mg. or more. Taylor and Brock (1934) proceed by wet oxidation of the material with sulphuric and perchloric acids and reduction of iron in the diluted solution with zinc in the usual way. The solution is then filtered with precautions against oxidation and the ferrous iron titrated with 0·01N permanganate.

Astruc and Castel (1935) determine iron directly in wine by reducing it with a thin suspension of cuprous oxide in dilute sulphuric acid. The cuprous oxide is prepared by boiling a copper sulphate solution with glucose. The reduced iron is titrated with permanganate. This is a variation of the method introduced by Bertrand in 1906 for the determination of cuprous oxide in sugar analysis.

IODIMETRIC DETERMINATION. Rappaport and Hohenberg (1934) employ an iodimetric method for traces of iron in foods. They use potassium cadmium iodide, K_2CdI_4, which is more stable than potassium iodide and which gives free iodine when boiled with a solution containing ferric iron. Iodine is distilled off and oxidised to iodic acid in the distillate with bromine. The iodic acid is then treated with excess of potassium iodide and the liberated iodine titrated with thiosulphate. In this way six equivalents of iodine are titrated for every equivalent of iron present, which increases the delicacy of the method. This method is of course applicable only when the solution contains no other substances capable of liberating iodine from potassium cadmium iodide.

COLORIMETRIC DETERMINATION WITH FERROCYANIDE. Walker (1925) gives a method of determining iron colorimetrically in the ash of foods with potassium ferrocyanide. He works with a solution of iron in dilute nitric acid, and finds that nitrous acid must be removed by boiling, otherwise a yellowish-green colour is obtained instead of Prussian blue. The sample is ashed, the ash extracted with concentrated nitric acid, the solution boiled to remove nitrous acid, diluted and treated with potassium ferrocyanide solution. The blue colour is then compared with standards.

This method is criticised by Mummery (1926), who finds that nitric acid does not take out all the iron from the ash of some foods, whereas hydrochloric acid does extract it all. He recommends that the extraction should always be carried out with hydrochloric acid, the solution being evaporated to dryness and then taken up with nitric acid for colorimetric determination by Walker's method.

Ribéreau-Gayon (1929) determines iron in white wines by direct colorimetry with potassium ferrocyanide without destroying organic matter.

COLORIMETRIC DETERMINATION WITH SALICYLIC ACID. Small amounts of iron can be determined by means of the purple colour given with salicylic acid. Mehlig (1938) and Scott (1941) have given the conditions under which the full development of colour is obtained. 100 ml. of the neutral solution are acidified with 10 ml. of diluted acetic acid (1 : 1), which should bring the acidity to pH 2·6–2·7, and a solution of sodium salicylate added. The colour is given only by ferric iron ; if ferrous iron is present it is oxidised by precipitation with a slight excess of ammonia and the precipitated ferric hydroxide redissolved in diluted acetic acid at the above strength. This method was worked out by Agnew (1928) for determining traces of iron in zinc and developed by Mehlig and by Scott for analysis of soils, etc. It does not seem to have been used for biochemical or food work, although a somewhat similar method using salicyl sulphonic acid has been given by Warren (1939 ;

cf. also Scott and Mitchell, p. 296). The colour is well adapted for measurement in a photoelectric absorptiometer, but has the disadvantage that it is affected by many commonly occurring substances, e.g. arsenates, nitrites, orthophosphates, pyrophosphates, oxalates, sulphites, tartrates, copper, manganese and tin. Scott has indicated the extent of interference by the commoner ions, and it would seem that in food the only ones likely to interfere seriously are phosphates.

MISCELLANEOUS METHODS. Among other methods of determining small amounts of iron are the following :

1. Colorimetrically as the dark-green complex with 8-hydroxyquinoline (Lavollay 1935).
2. Colorimetrically with 7-iodo-8-hydroxyquinoline-5-sulphonic acid, $C_9H_4N(OH)ISO_3H$ (Clark and Sieling 1936, Yoe and Hall 1937). This substance gives a permanent green colour with ferric iron at pH 2·7 to 3·2.
3. Reduction of ferric to ferrous salt with metallic copper in phosphoric acid solution, followed by reduction of phosphomolybdic acid by the ferrous salt and titration of the reduced phosphomolybdic complex with permanganate (Fontès and Thivolle 1923).
4. Reduction of ferric iron with finely divided silver (silver reductor) and titration with ceric sulphate solution using *o*-phenanthroline as indicator (Walden, Hammett and Edmonds 1934, Willard and Young 1928).
5. Precipitation with the ammonium salt of nitrosophenylhydroxylamine or ‘cupferron’ (Baudisch 1909).

Several other methods for the micro-determination of iron in biological material and foods are referred to in a list of collected references by Stary (1933).

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9

[NICKEL]

NICKEL does not, as far as is known, play any part in human or animal nutrition or in plant growth and no diseases of plants or animals have been attributed to the lack of it. Its interest to the food chemist arises from its frequent use as the pure metal, as nickel plate, or as a constituent of alloys in the construction of plant and vessels in food industries and for cooking, and also as a metallic catalyst in the hydrogenation of edible oils.

NICKEL IN SOILS. Schieckenthal (1927) has given the nickel and iron content of a number of ferruginous soils near Vienna. The nickel content averaged about 0·004 per cent (40 p.p.m.) and the iron content about 4 per cent. Bertrand (1922) found traces of nickel in all soils examined, up to 17 p.p.m., and Russian analyses have also shown amounts ranging from 2 to 14 p.p.m. in different soils. Probably only a small fraction of this is in a condition in which it could be absorbed by plants, as measured by solubility in dilute acetic acid (Scott and Mitchell 1943). Mitchell (1944) has given a review of spectrographic work on the distribution of nickel, cobalt and a number of other trace elements in Scottish soils.

Excess of nickel is toxic to plants. Certain soils on serpentine outcrops in Maryland and Pennsylvania are stated to owe their lack of fertility to the presence of relatively large amounts, over 0·1 per cent, of nickel and chromium salts.

NICKEL IN PLANTS. Very few determinations have been made of nickel naturally occurring in plants. Bertrand and Mokragnatz (1930) found that the mean nickel content of a number of vegetables, fruits and grains (calculated on the dry material) was about 0·9 p.p.m. as against only 0·04 p.p.m. of cobalt. Lettuce, cabbage, spinach and peas contained from 1·5 to 3·0 p.p.m., wheat 0·35 and fruits 0·15 to 0·25 p.p.m. In potatoes they found 0·25 p.p.m. of nickel and 0·06 p.p.m. of cobalt. Scott and Mitchell found from 0·56 to 3·62 p.p.m., calculated on the dry matter, in various pasture samples from north-east Scotland. According to Ramage (1936) nickel occurs in a number of spices and herbal drugs and is also present in tea. He found as much as 0·014 per cent in *Strychnos Ignatii* beans.

NICKEL IN HUMAN AND ANIMAL ORGANS. Bertrand and Machebœuf (1925) found that human and animal organs contain as a rule far less nickel than cobalt, in contrast to plants. Fresh human liver contained 0·09 and pancreas 0·04 p.p.m. of nickel, and ox liver and pancreas 0·125 and 0·135 p.p.m. respectively. They found 0·004 p.p.m. in cows' milk and 0·02 p.p.m. in egg-yolk. Fish contained on an average about 0·015 p.p.m. Fresh lobster showed 0·1 p.p.m. of nickel and 2 p.p.m. of cobalt, but in mussels the nickel predominated, being 0·45 p.p.m. as against 0·14 p.p.m. of cobalt.

NICKEL IN FOOD. Small quantities of nickel may be found in various manufactured foods. In edible gelatin, according to Martini (1929) and Maclaurin (1932), it occurs rather frequently owing to its presence in traces as a normal constituent of bones. It also occurs in hardened oils which have been in contact with a nickel catalyst. The amount found in such oils varies considerably, from barely perceptible traces of the order of 0·01 p.p.m. to as much as 1 or 2 p.p.m. Some hardened oils give a strong magenta colour on heating and shaking with dilute hydrochloric acid and adding a small quantity of potassium dithio-oxalate solution (p. 280), but with others this test may fail unless a large sample is extracted and the extract concentrated.

Nickel salts may gain access to food from corrosion of nickel vessels. Cox (1935) states that a beverage brewed in nickel contained 60 p.p.m., equivalent to about 2 grains of crystallised nickel sulphate per pound, which corresponds closely to the amount of copper sulphate formerly used for greening vegetables. Lehmann (1908) found that food cooked in nickel utensils took up from 10 to 58 p.p.m., and he calculated that if nickel ware was used exclusively in a kitchen the total amount of nickel ingested daily would be about 117 mg. or 1·5 to 2 mg. per kilogram of the consumer's weight. This he considered negligible. Normann and Hugel (1913) found from 19 to 83 p.p.m. in apples and vegetables cooked in nickel vessels and as much as 127 p.p.m. in sauerkraut. According to Ludwig (1898) food cooked in nickel contained from nil to 35 p.p.m. and sauerkraut 129 p.p.m. Dzerzgowsky (1906) found that solutions of various organic acids up to 4 per cent strength, with and without salt, when boiled in nickel for 3 hours and allowed to stand for a further 3 to 11 hours, took up 60 to 320 p.p.m. of nickel. According to Järvinen (1923) a 40 per cent sugar solution containing 1·5 per cent of citric acid, boiled for 3 hours in a nickel vessel, took up 76 p.p.m. ; a 5 per cent salt solution under similar conditions took up only 5 p.p.m., but salt in presence of organic acids is more corrosive. Nickel is corroded even at room temperature by citric acid solutions in presence of oxygen. Johnston (1935) records that a 66 per cent sucrose solution containing pineapple chunks, allowed to remain in contact with sheet nickel for six weeks, took up an amount corresponding to about 43 p.p.m. Hagues (1931) found that in nickel-lined fermentation vessels nickel was corroded where it came into contact with the yeast head and the yeast contained up to 140 p.p.m. of nickel. The beer contained only about 2 p.p.m.

Food cooked in nickel vessels may become more or less discoloured and have a metallic taste. 5 to 10 p.p.m. will give a taste to fruit, etc., and 14 p.p.m. of nickel lactate in water has a decidedly metallic taste. Charley (1933) states that nickel vessels do not affect the flavour of cider. Nickel and iron salts have been found to turn tomato products brown.

According to some authorities nickel is eminently suitable for dairy plant and is more resistant to corrosion than aluminium and tin (Donauer 1922, Searles 1930). Milk pasteurised under commercial conditions in nickel apparatus does not contain more than 0·25 to 1·3 p.p.m. Other authorities, however, assert that milk if pasteurised in nickel vessels may take up as much as 12 to 15 p.p.m., and may acquire a metallic taste from the presence of nickel lactate (McKay and others 1929, Pratt 1930, Burrell 1938). Quam (1928) found that milk agitated with metal strips at 75° for 30 minutes took up 6 to 7 mg. of nickel per dm.^{2} of surface as against 1·5 mg. of copper and 0·85 mg. of zinc. Nickel appears to be more suitable for surface preheaters in continuous pasteurisation than for surface coolers, since a protective film of milk protein is formed on the surface of hot nickel in contact with cold milk, while no such film is formed by cold metal in contact with hot milk. Génin (1935) found that corrosion of nickel in condensed milk evaporators was greater during intermittent than during continuous working. Nickel salts inhibit bacterial action, and it has been stated that 11 p.p.m. delays lactic fermentation in milk, but it seems doubtful whether such a small amount would have any appreciable effect.

According to Hartley (1935) steam-jacketed nickel pans are used for processing pineapples, cranberries, soups, jams, dairy produce, vegetables, etc. Nickel does not appear to act as an oxidation catalyst for vitamin C. It is attacked less easily than aluminium and tin by alkaline detergents and brines.

Of the alloys of nickel, monel metal (Ni 67, Cu 28, Fe and Mn 5 per cent) is more resistant than nickel and is widely used in plant and apparatus in food industries. It resembles pure nickel in its resistance to brine products (Tay 1936), but Génin (1938) finds that it is attacked by salt solutions unless small amounts of calcium hydroxide or sodium chromate are present, which protect the metal from corrosion. In brewery fermentation vessels monel metal is about five times as resistant as nickel. Inconel metal (Ni 80, Cr 14, Fe 6 per cent) is still less liable to corrosion and tarnishing and is a most suitable metal for dairy plant. Titus and others (1930) have investigated the resistance shown by nickel-chromium-iron alloys to various foods during cooking and storage. The average amounts dissolved in one hour's cooking in contact with an alloy containing 15 per cent of nickel and 18 per cent of chromium were 1 mg. of iron, 0·05 mg. of chromium and 0·06 mg. of nickel from 4 dm.^{2} of surface. White nickel bronze (Cu 75, Ni 25 per cent) is stated to be readily corroded and to be unsuitable for cooking vessels.

TOXICITY OF NICKEL. Nickel compounds are similar in their effects to those of copper and zinc. According to Dzerzgowsky (1906) doses of 10 to 20 mg. of nickel per kilogram cause no symptoms in dogs, but 22 to 44 mg. per kilogram produce vomiting and diarrhoea. 2 to 5 gr. of the sulphate (containing 31 to 67 mg. of nickel) may cause nausea and vomiting in human beings (Kolipinsky 1911), whereas the emetic dose of copper sulphate is given as 5 to 10 gr. (80 to 160 mg. of copper) and of zinc sulphate 10 to 30 gr. (147 to 445 mg. of zinc), from which it would seem that nickel sulphate is a more severe stomach irritant than the sulphates of copper or zinc. One cannot, however, assume that the action of these metals in combination with food acids is comparable with that of irritant salts like the sulphates. Even ferrous sulphate has a medicinal dose corresponding to only 13 to 65 mg. of iron. Organic salts of these metals are as a rule much less irritant. 180 mg. of nickel in the form of acetate has been given to a dog daily for a month with only slight diarrhoea and loss of appetite, and the dog gained in weight. According to Arnold (1939) a daily dose of 80 mg. of nickel in the form of nickel pectinate given to young rats, corresponding to 1·25 g. per kilogram of body weight, did not affect their rate of growth over a period of 8 weeks.

When administered by the mouth in subemetic doses no ill effects have been observed either in animals or man, and Dzerzgowsky (1906) concluded that if the alimentary tract is intact the metal is wholly excreted in the faeces without absorption. If the mucous membrane is injured nickel may be absorbed. Flinn and Inouye (1929) also found that it was excreted to the extent of 98 to 99 per cent by the intestine and not by the kidneys. Kent and McCance (1941), on the other hand, in an experiment on two normal men with very low intakes of nickel in food (0·3 to 0·5 mg. daily), found that roughly two-thirds of the nickel was excreted in the urine and only one-third in the faeces. Their experiment did not show what would be the proportion at higher rates of intake.

In 1886 the use of nickel for cooking vessels was forbidden in Austria, but the decree was rescinded in 1897. Several investigators have recorded that their families have used nickel vessels exclusively in the kitchen for a number of years without any ill effects whatever, but negative evidence of this kind does not exclude the possibility that they might have been still better without nickel. Dzerzgowsky suggested that continued ingestion of subtoxic doses of nickel salts dulls the nerves of the alimentary tract, making it possible for more metal to be given without irritation, but there is no evidence of this. It is stated that Offerdahl in 1913 took 0·5 g. of powdered metallic nickel daily for a month without ill effects.

Nickel salts appear to be more toxic when injected into the blood-stream. Stuart in 1882 found that rabbits and dogs were particularly susceptible and were killed by 7 to 8 mg. of nickel sodium citrate per kilogram, death being due to cardiac paralysis and toxic action on the central nervous system. Caujolle and Canal (1939) found that 10 to 20 mg. of nickel per kilogram injected into dogs proved fatal, while the corresponding dose of cobalt was 30 mg. per kilogram, indicating that nickel if it gains access to the circulation is the more toxic of the two.

According to Richet (1881) fish are less sensitive to nickel than to copper, zinc, iron or cadmium and can live for more than 48 hours in water containing 125 p.p.m.

A full discussion of the hygienic significance of nickel is given by Drinker and others (1924) with many references on the subject, and there are also reviews by Schreiber (1934, 1935).

It is difficult to suggest practical limits for nickel in foods. It is rarely found in foods in amounts comparable to those of zinc, since it is far more resistant than zinc to oxidation and corrosion. Moreover, nickel is cathodic to iron, whereas zinc, being anodic to iron, dissolves freely from galvanised iron vessels in contact with acid liquids. There are no records of any ill effects being caused by the ingestion of subemetic doses of nickel, but the same is true of zinc salts and it is by no means certain that irritant substances of this kind even in small doses are wholly without effect on health. On the other hand, there does not seem to be any strong evidence that nickel when ingested is more toxic than iron. The action of nickel salts on animals seems to resemble that of zinc salts. Both metals are excreted mainly in the faeces and the effect, in similar doses, is primarily that of a stomach irritant. It would seem that the doses of each required to produce toxic effects, either as irritants or through absorption from the alimentary tract, may be roughly similar. Pending further knowledge of the absorption and physiological action of nickel, provisional limits might be of the same order as those for zinc, that is about four times the limits appropriate to copper (pp. 35–8). The possibility that some persons may be specially susceptible to nickel poisoning is suggested by the frequent occurrence of nickel dermatitis among factory workers from contact of the skin with nickel salts. It is said that even white-gold spectacle frames containing nickel have been known to cause dermatitis (Cormia 1935). Stewart (1933) records the case of a patient whose skin was sensitive to nickel and cobalt but not to iron.

NICKEL AND INSULIN. Salts of nickel, in common with those of certain other metals, reinforce and sustain the hypoglucaemic action of insulin when added in small amounts (0·05 to 4 mg. per 100 units), but in larger amounts they completely inhibit its action (Sahyun and others 1939, Schwab 1938). A crystalline nickel insulinate can be prepared containing 0·41 per cent of nickel (Fischer and Scott,

p. 110). There is no reason to suppose, however, that the physiological action of insulin is in any way dependent on the presence of nickel.

THE DETERMINATION OF NICKEL IN FOOD

WITH DIMETHYL-GLYOXIME. This reagent was introduced by Tschugaeff in 1905. It has the formula $CH_3.CNOH.CNOH.CH_3$ and its alcoholic solution gives with neutral solutions of nickel salts a red crystalline precipitate, $[CH_3.CNOH.CNOH.CH_3]_2Ni$. This compound is soluble in mineral acids but practically insoluble in neutral or acetic acid solutions. The precipitate can be dried at 100° to 120° and weighed. On being heated to 250° it sublimes without decomposition. With small amounts of nickel the colour of the liquid containing the red precipitate in suspension is compared with that of standards, or the precipitate is filtered through a paper disc and the depth of colour of the deposit compared with that of standards filtered through similar paper discs.

Von Fellenberg (1940) in examining hardened fats for nickel proceeds as follows. 10 g. of the fat are heated in a 100-ml. conical flask with 10 ml. of hydrochloric acid (6 parts of concentrated acid and 4 of water) for 30 minutes, during which a current of air is driven through the liquid to ensure thorough and continuous agitation. Without this air current or alternatively constant shaking, nickel may not be completely extracted from the fat by the acid. The aqueous liquid is then filtered through a wet cotton-wool plug. The filtrate is concentrated to a small bulk, made alkaline with ammonia, again brought to the boil and filtered through a plug of paper pulp in a small filter funnel. This filtration removes any iron hydroxide present. The clear filtrate, which may be yellowish in colour, is treated with one drop of a 1 per cent alcoholic solution of dimethyl-glyoxime, and after being allowed to stand for 2 minutes is filtered through a small wad of filter-paper pulp. The wad of paper is washed with water and the intensity of the red colour on its surface compared with that of standards. Von Fellenberg claims that the test will detect as little as 0·001 mg. of nickel in 10 g. of fat, but it would seem that this degree of sensitiveness can be reached only if the solution to be tested has been concentrated to a very small volume, since Strafford (1923) gives the sensitiveness of the glyoxime precipitation as not more than 1 in 500,000. Torricelli (1937) gives a somewhat similar method.

Struszynski (1935) gives the following test for nickel in hardened fats. 100 to 200 g. of the fat are melted and filtered clear, an equal volume of concentrated hydrochloric acid is added, together with one drop of nitric acid, and the flask is heated on a steam bath with shaking. The acid layer is separated and evaporated to dryness, and the residue ignited and dissolved in 2 to 3 drops of a mixture of hydrochloric and nitric acids. The solution is diluted to 2 ml., a small quantity of tartaric acid added and aqueous ammonia until the liquid is faintly alkaline, followed by a 1 per cent solution of dimethyl-glyoxime in alcohol. The liquid is extracted with chloroform and the chloroform solution evaporated to dryness. A red spot or ring indicates the presence of nickel. Iron does not interfere. The test is claimed to be extremely sensitive.

Armit and Harden (1905) determined nickel in organic material by dry ashing and removing iron and phosphate with ammonia, and copper with hydrogen sulphide. From the filtrate nickel is precipitated with sodium hydroxide and converted into nickel chloride and the solution made up to a definite volume in a Nessler glass. Nickel is then determined colorimetrically with dimethyl-glyoxime. The method is applicable to rather larger amounts of nickel than those referred to above. Fairhall (1926) criticises this method, with which he was unable to obtain more than a rough approximation. Passamaneck (1945) dissolves the nickel compound of dimethyl-glyoxime in pyridine and compares the colour of the clear yellow to orange-red solution with that of a standard nickel solution in a Duboscq micro-colorimeter.

Other α-dioximes give similar reactions with nickel, and Atack (1913) recommended α-benzil-dioxime as having decided advantages over dimethyl-glyoxime. It gives with nickel salts an intense red precipitate analogous in composition to that given by dimethyl-glyoxime. The sensitiveness is given as 1 part of nickel in 2,000,000 of water or 0·0025 mg. of nickel in a volume of 5 ml. The test is not interfered with by zinc, whereas large amounts of zinc may prevent the formation of the dimethyl-glyoxime precipitate. Atack used this method for the determination of fairly large quantities, 4 mg. or more, of nickel, the precipitate being weighed on a tared filter-paper. It contains 10·93 per cent of nickel. The method can be adapted to the determination of smaller quantities as with dimethyl-glyoxime. When so used it has the advantage that the colour is not so likely to be affected if cobalt is present.

DETERMINATION WITH POTASSIUM DITHIO-OXALATE. This reagent was introduced by Jones and Tasker (1909). It has the formula SK.CO.CO.SK and is a white powder which becomes brown on keeping. It is readily soluble in water to a yellow solution which gives a deep magenta colour with traces of nickel. Jones and Tasker state that it will detect 1 part of nickel in 40,000,000 parts of water if enough of the solution is used to fill a large beaker, and that 1 part in 8,000,000 can be detected readily in a test-tube. The colour given with cobalt (deep brown) is just as intense as that given with nickel. The nickel colour is slowly destroyed by dilute hydrochloric acid and quickly by potassium cyanide. The cobalt colour, on the other hand, is stable in dilute hydrochloric acid and also in cyanide solution. Cobalt can therefore be detected in presence of nickel by adding cyanide to the solution, when the magenta colour disappears and the brown due to cobalt remains. Copper, even at dilutions of 1 in 1,000,000, gives a yellow or greenish-yellow colour, and iron a purplish colour, but only in solutions stronger than 1 in 100,000.

Potassium dithio-oxalate is a better reagent than dimethylglyoxime for the colorimetric determination of nickel in that the coloured compound is soluble, whereas nickel glyoxime is an insoluble solid in suspension and the size of the particles and therefore the depth of colour are liable to be affected by salt concentration and other conditions of precipitation. A further advantage is that the colour is developed in neutral or weakly acid solution, which makes it possible to determine nickel without removal of calcium, magnesium and phosphates. Potassium dithio-oxalate has the disadvantage that it is not so specific as dimethyl-glyoxime. Further, it is not very stable unless kept under absolute alcohol and is not usually obtainable commercially. Its preparation by the following method (Jones and Tasker 1909), although not difficult, is an unpleasant operation owing to the offensive and persistent smell of ethyl mercaptan.

Oxalyl chloride is first prepared by the action of 2 mols. of phosphorus pentachloride upon 1 mol. of anhydrous oxalic acid, the mixture being kept cold with ice. It is allowed to stand for two or three days at room temperature until the whole mass is liquid, and oxalyl chloride, which boils at 64°, separated from phosphorus oxychloride by fractional distillation. To prepare ethyl dithio-oxalate, oxalyl chloride (1 mol.) is added carefully drop by drop at room temperature to ethyl mercaptan (2 mols.). The liquid becomes yellow and hydrogen chloride is evolved. The mixture is heated gently to expel any excess of either of the reacting substances, and on cooling sets to a crystalline mass consisting of almost pure ethyl dithio-oxalate. This is dissolved in alcohol and treated with an equivalent quantity of alcoholic potassium hydrosulphide (prepared by passing hydrogen sulphide into an alcoholic solution of potassium hydroxide until the cold solution is saturated with the gas). On stirring the mixture vigorously and allowing it to stand for about an hour potassium dithio-oxalate is obtained as a white crystalline precipitate. This is filtered off and washed free from potassium hydrosulphide with alcohol. The salt is best kept under absolute alcohol in a well-corked bottle in a dark place:

under these conditions it can be kept for years without appreciable deterioration.

Fairhall (1926) gives the following directions for the colorimetric determination of nickel with potassium dithio-oxalate. The material is ashed in a muffle and the ash dissolved in hydrochloric acid. The solution is neutralised to methyl orange with ammonia solution and made faintly acid with hydrochloric acid. Any heavy metals present are removed with hydrogen sulphide, and the filtrate is boiled and treated with bromine water to oxidise iron. The liquid is cooled, and iron is removed as phosphate by adding ammonium acetate solution followed by a few drops of acetic acid. The filtrate, which should be clear and colourless, is diluted to a known volume, say 500 ml. 50 ml. of this solution are transferred to a Nessler cylinder and a small amount of potassium dithio-oxalate solution added. If nickel is present a clear magenta colour develops at once, which is compared with a series of nickel standards containing 0·005 to 0·05 mg. in 50 ml. In absence of nickel the solution may be slightly yellow from excess of dithio-oxalate. If iron has not been completely removed the colour may be purplish, and the determination must be repeated with precautions to remove all iron.

If appreciable amounts of cobalt are present, the reddish-brown colour of the cobalt complex may interfere with the determination of nickel. Drinker (1924) recommends a preliminary separation of nickel from cobalt by precipitation with α-benzil-dioxime. Iron is first de-ionised with sodium citrate, calcium is removed as oxalate and magnesium as magnesium ammonium phosphate. Nickel is then precipitated in slightly ammoniacal solution with α-benzil-dioxime, the precipitate oxidised with aqua regia and the nickel determined colorimetrically with potassium dithio-oxalate. This method is long and troublesome, and it should be possible to base a colorimetric method on the behaviour of the nickel and cobalt complexes in the presence of cyanide referred to above.

Dithio-oxamide, or rubeanic acid, which also forms a violet nickel compound, has the formula $S{:}C(NH_2).C(NH_2){:}S$, or in its acid tautomeric form $SH.C(NH).C(NH).SH$. It is therefore closely related in constitution to dithio-oxalic acid, but has the disadvantage that it gives a black colour with extremely small amounts of copper which may mask the colour due to nickel.

Separation of Nickel and Cobalt from other Metals with Dithizone. Strafford, Wyatt and Kershaw (1945), using a solution of dithizone in toluene, find that the nickel and cobalt complexes with dithizone are stable in presence of a buffered N/10 solution of hydrochloric acid at pH 1·5, and that these metals can thus be separated from lead and zinc. In the table on page 131 nickel and cobalt are shown as not reacting in acid solution with dithizone in chloroform. The dithizone complexes of these metals appear to be formed with difficulty in presence of acid, so that for practical purposes they cannot easily be extracted from acid solutions with dithizone dissolved in chloroform. When once formed, however, by extraction from neutral or slightly alkaline solutions they are not readily decomposed by shaking the chloroform solution with dilute acid.

Young and others (1946) determine nickel by treating the solution first with dimethyl-glyoxime and extracting the resulting nickel glyoxime with chloroform. This separates nickel from all other metals likely to be present. The chloroform solution of the glyoxime is shaken with 2 per cent hydrochloric acid, and nickel thus transferred to the acid solution. This solution is neutralised and brought to pH 8 with ammonia, and the nickel determined by titrimetric extraction with a solution of dithizone in carbon tetrachloride, which has previously been standardised against a nickel solution. The nickel compound with dithizone is purple in carbon tetrachloride solution.

Spectrographic Method. Scott and Mitchell's spectrographic method (1943) for cobalt, nickel and molybdenum in soils and plants is referred to on page 295.

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10

[COBALT]

COBALT is one of the elements shown to be essential in the nutrition of certain domestic animals, although it has not yet been proved to be so for man.

COBALT DEFICIENCY IN ANIMALS. In several parts of the world cattle and sheep are liable to suffer from a wasting disease known as enzoötic marasmus or Denmark disease (West Australia), coast disease (South Australia), bush sickness or Morton Mains disease (New Zealand), salt sickness (Florida), nakuruitis (Kenya) and 'pine' in the Cheviot district of the Scottish border, the island of Tiree in the inner Hebrides and on Dartmoor. The disease is associated with an anaemic condition and is characterised by lethargy, loss of weight, quick pulse and respiration, loss of milk production, absence of oestrum, and abortion. The first indication that this might be due to some abnormality in the pasturage appears to have been given as long ago as 1807 by James Hogg, the Ettrick shepherd, who referred to it as a 'distemper . . . affecting sheep confined to some districts . . . but removing them to fine land cures them immediately'. It was for some time supposed to be due to lack of iron, since it could be cured or prevented by giving the animals a salt lick containing limonite (brown haematite). Some samples of limonite, however, had no effect, and it was obvious that iron alone was not the curative agent. A series of experiments by Underwood and Filmer in West Australia in 1935 showed that the active agent was cobalt (cf. also Lines 1935, Marston 1935). The inefficacy of some samples of limonite was due to the fact that they contained no cobalt, or at any rate less than 50 p.p.m., instead of the normal amount of 200 to 300 p.p.m. Cobalt had been suspected at least ten years previously (Bertrand and Machebœuf 1925) of being concerned in biological processes, owing to its wide distribution in animals and its action in producing polycythaemia (cf. Stare and Elvehjem 1933).

It is probable that copper is associated with cobalt in the prevention of coast disease. Certain localities seem to be deficient in both copper and cobalt, since the administration of cobalt alone, or of copper alone, to diseased animals will not effect a cure (Marston and others 1938). The extent to which copper and cobalt are interdependent is still rather obscure.

COBALT CONTENT OF SOILS AND PASTURES AND ANIMAL REQUIREMENTS. Soils vary widely in their cobalt content. The New Zealand soils examined by Kidson (1937) varied from 0·3 to 40 p.p.m. with occasional samples showing much more, but only a small proportion of this is available to plants as measured by solubility in dilute acetic acid (Scott and Mitchell 1943). If the cobalt content falls below about 5 p.p.m. of the dry soil cattle and sheep are likely to show wasting, whereas on soils containing from 5 to 40 p.p.m. they remain healthy. Dartmoor soils on which sheep suffer from deficiency contain from 1·9 to 6·7 p.p.m. of cobalt. If moved to lowland soils containing 13 to 33 p.p.m. the sheep recover. A dressing of about 2 lb. of cobalt salt per acre is sometimes enough to prevent ‘ pine ’.

The pasture on deficient soils contains less than 0·07 p.p.m. of cobalt on the dry basis, while that grown on healthy soils contains from 0·07 to 0·3 p.p.m. or occasionally up to 1·0 p.p.m. What proportion of this is available for metabolism is not known, but probably most of it is utilised. If we take it that a full-grown sheep eats about 3 lb. (dry weight) of grass daily, this places the minimum cobalt requirement at the extremely small amount of 0·1 mg. Wunsch (1937) calculated that on an average cobalt-deficient pasture, one ounce of additional cobalt, used without waste, would be enough to keep a flock of 1,400 sheep healthy for a year.

In practice, 1 mg. daily in the form of a soluble cobalt salt is given to sheep and cattle as a curative dose (Corner and Smith 1938). Apparently it is only ruminants which are affected by cobalt deficiency ; horses and pigs do not seem to be so susceptible.

COBALT IN HUMAN AND ANIMAL ORGANS. The livers of sheep suffering from cobalt deficiency contain on an average 0·04 p.p.m. on the dry basis, as against 0·2 to 0·3 p.p.m. in healthy livers (Underwood and Elvehjem 1938, McNaught 1939). The livers of normal rats contain about the same proportion, 0·2 p.p.m. on the dry weight (McNaught 1939). Since a rat's liver weighs only 1 g. when dry, the total amount of cobalt is extremely small, about 0·2 μg. Bertrand and Macheboeuf (1925) have given the figure 0·25 p.p.m. for human kidney and liver (fresh), corresponding to 1·3 and 0·9 p.p.m. respectively for the dry substance, and still higher values for spleen (0·47 p.p.m. fresh, 2·6 p.p.m. dry) and pancreas (0·35 p.p.m. fresh, 2·0 p.p.m. dry). Dutoit and Zbinden (1930), however, could not find cobalt in human liver by the spectroscope, but only in the pancreas. Bertrand and Macheboeuf also found that in farm animals the pancreas is richer in cobalt than any other organ, the amounts found being 0·18 p.p.m. in pig, 0·34 p.p.m. in sheep, 0·35 p.p.m. in calf and 0·5 p.p.m. in horse pancreas, all on the dry material. Ahmad and McCollum (1939) found about 0·2 p.p.m. in the dried pancreas of farm animals. Neal and Ahmann (1937) calculate that a cow weighing 450 kg. normally has 25 to 30 mg. of cobalt in its body and that its daily intake in food is 1 to 2 mg.

Bertrand and Macheboeuf (1925) concluded that in plants there is, as a rule, more nickel than cobalt, whereas human and animal organs contain more cobalt than nickel, indicating a preferential absorption of cobalt in the alimentary tract.

EXCRETION OF COBALT. According to Askew (1936), cobalt is rapidly absorbed by sheep and eliminated both in urine and faeces, and Mascherpa (1927) found that when given by the mouth half is excreted by the kidneys and half in the faeces. Greenberg and others (1943) found that with minute doses of 0·1 mg. administered by the mouth to rats about 18 per cent was excreted in the urine and about 40 per cent in the faeces. Kent and McCance (1941) suggest that it is not readily absorbed in man and that most of it is eliminated in the faeces.

COBALT AND POLYCYTHAEMIA. When cobalt is given to animals in excess of their normal requirement, but still in amounts which appear by no means excessive, it produces polycythaemia (increase in the number of red corpuscles in the blood). Thus 0·5 mg. daily given to rats (Orten and others 1932), resulting in an accumulation of only 0·04 to 0·05 mg. in the whole body, is enough to produce decided polycythaemia, but no increase in haemoglobin (Stare and Elvehjem 1933). Larger doses may cause intense polycythaemia and loss of weight (Josland 1936). The lethal dose for rats is given as 20 mg. per 100 g. body weight (Smith 1943). In dogs 2 mg. of cobalt per kilogram of body weight given daily for 2 weeks caused a 20 per cent increase in the number of red cells, but no further toxic symptoms were produced by three times this dose (Davis 1937). Similar effects have been obtained in other laboratory animals such as frogs, mice and guinea-pigs by injection of minute amounts of cobalt, and mild polycythaemia can be produced in sheep by moderately large doses. Barron (1937) suggested that the polycythaemic action of cobalt might be due to an inhibition of the respiratory functions of the red cells. These cells are then thrown into the circulation as imperfect non-respiring cells and are replaced in the bone marrow by new cells. Orten and others (1933) determined the relative volumes of blood plasma and erythrocytes in rats with cobalt polycythaemia, with the object of ascertaining whether this was relative, due to decrease in blood volume; apparent, due to redistribution of inactive cells; or true polycythaemia due to active addition of erythrocytes. They came to the conclusion that cobalt, provided that copper is also present, produces a true polycythaemia. The association of copper with cobalt in the treatment of coast disease has been referred to above. Neal and Ahmann (1937) found that the anaemia produced in calves by lack of cobalt was characterised by abnormally small erythrocytes and low colour index. Cobalt has not, however, been associated, like copper, with anaemia therapy in man. Underwood and Elvehjem (1938) found, also, that it plays no part in the treatment of anaemia in rats caused by a milk diet.

Apart from this tendency to produce polycythaemia, cobalt salts appear to be only moderately toxic. The lethal dose of cobaltous oxide when injected into small animals in the form of a soluble citrate was found by Stuart in 1882 to vary from about 0·01 to 0·14 g. per kilogram. For dogs the lethal dose of cobalt when injected is more than 0·03 g. per kilogram (Caujolle 1939).

Griffith and others (1942) find that cobalt combines with thiol compounds and consequently inhibits oxidation mechanisms. The toxic symptoms and loss of weight observed when 0·12 per cent of cobalt sulphate is added to a rat's food are largely counteracted if additional thiol compounds (e.g. cysteine) are added to the diet (cf. pp. 11 and 163).

COBALT AND INSULIN. Bertrand (1926) found that cobalt as well as nickel increased the hypoglucaemic action of insulin. This is analogous to the effect of zinc (p. 110). The action of cobalt in promoting the crystallisation of insulin, and the preparation of a crystalline cobalt insulin containing 0·44 per cent of cobalt are of interest in this connection. It would appear that several metals may combine with insulin and influence its action in controlling blood sugar.

COBALT IN FOODS. Comparatively few determinations of cobalt in foods have been made. Bertrand and Mokragnatz (1930) found amounts varying from the merest trace up to 0·35 p.p.m. in the dry matter of vegetables, fruits and cereals, the highest occurring in lentils and buckwheat. Grimmett (1937) found 0·003 p.p.m. in corn starch, 0·004 to 0·007 p.p.m. in cod-liver oil, lard and wheat germ oil, 0·013 p.p.m. in butter and 0·038 in yeast. Sylvester and Lampitt (1940) found figures of a similar order for white flour (0·003 p.p.m.) and wholemeal flour (0·01 p.p.m.). They also found about 0·15 p.p.m. in tea, 0·04 p.p.m. in coffee beans, 0·3 p.p.m. in cacao beans, and 0·001 p.p.m. in milk.

Ahmad and McCollum (1939) found from 0·2 to 0·5 p.p.m. in beans and peas of various kinds, 0·07 p.p.m. in maize, 0·06 to 0·15 in wheat and 0·06 to 0·07 in milk powder, all on the dried materials. Their figures for wheat and milk are thus considerably higher than those of Sylvester and Lampitt. Hurwitz and Beeson (1944) give the cobalt contents of a number of foods calculated on the dry basis.

In considering the cobalt assumed to occur naturally in foods it should be borne in mind that cobalt compounds are often used to colour the vitreous enamel of hollow-ware, and are also a common constituent of the ground coat which underlies the finished enamel, cobalt silicates having been found to adhere well to steel plate: traces of cobalt may perhaps gain access to food from this source. Cobalt driers (e.g. cobalt linoleate) are also often used in the preparation of lacquers for cans, and this may conceivably result in traces of the metal being found in canned foods. Cobalt driers are also met with in the paints sometimes used on the inner surfaces of fish boxes.

THE DETERMINATION OF COBALT IN FOOD

DETERMINATION AS COBALTINITRITE. Bertrand and Mokragnatz (1925) used the potassium cobaltinitrite reaction for the detection and approximate determination of cobalt in plants, but the method is not highly sensitive since potassium cobaltinitrite is slightly soluble. Caesium cobaltinitrite is more insoluble and the double cobaltinitrite of caesium and potassium still more so. It is claimed (Yagoda and Partridge 1930) that a solution containing 0·01 mg. of cobalt per millilitre will give a precipitate of caesium potassium cobaltinitrite in about 3 minutes. The ash of a substance suspected to contain cobalt is dissolved in 1 or 2 ml. of 30 per cent acetic acid. To this solution are added 2 ml. of 40 per cent potassium nitrite solution and 0·5 ml. of 10 per cent caesium nitrate solution. The yellow precipitate is stated to have the composition $Cs_2KCo(NO_2)_6$. Considerable amounts of iron, manganese and nickel do not interfere with the reaction. The precipitate may be oxidised with standard permanganate solution, and the excess of permanganate determined with potassium iodide and thiosulphate (Wassilieff 1929).

COLORIMETRIC DETERMINATION WITH DIMETHYL-GLYOXIME. Dimethyl-glyoxime is primarily a reagent for nickel with which it gives a red crystalline precipitate. Cobalt gives a yellowish-brown colour and can be determined colorimetrically after removal of nickel. The filtrate from the precipitation of nickel is evaporated to dryness and the residue heated to destroy organic matter. It is dissolved in hydrochloric acid, the solution evaporated and the residue taken up with about 2 ml. of water. Sufficient tartaric acid is added to prevent interference from iron, and the solution is made slightly alkaline with ammonia. On addition of an alcoholic solution of dimethyl-glyoxime a yellowish-brown colour is produced if cobalt is present. According to Bertrand the colour is perceptible with 0·01 mg. of cobalt in 2 ml. of water. Copper gives a colour similar to that of cobalt in ammoniacal solution, and must therefore be removed as sulphide.

COLORIMETRIC DETERMINATION WITH NITROSO-R-SALT. In 1885 Ilinsky and von Knorre introduced α-nitroso-β-naphthol as a precipitating agent for cobalt, and it has been used by Agnew (1928), Mayr and Feigl (1932) and Phillipot (1935) for cobalt separation in inorganic analysis. Solutions of α-nitroso-β-naphthol do not keep well and are apt to produce a precipitate even in absence of cobalt, which makes it necessary to confirm the presence of cobalt in the precipitate by other methods. The closely allied β-nitroso-α-naphthol gives similar insoluble compounds with cobalt and some other metals.

Van Klooster (1921) found that the sodium disulphonate of α-nitroso-β-naphthol, or nitroso-R-salt, is a much better reagent. It is prepared by treating sodium 2-naphthol-3:6-disulphonate (R-salt) with nitrous acid. Nitroso-R-salt forms stable yellow crystals fairly soluble in hot water but only slightly in cold (100 ml. dissolves 2·6 g.). It gives an intense red colour with cobalt solutions, forming the compound $[C_{10}H_4OH.NO(NaSO_3)_2]_3Co$. This compound is stable in boiling dilute nitric acid, while the corresponding compounds with iron, nickel, copper and zinc are unstable, although the colours due to iron and copper are not completely removed by nitric acid.

Stare and Elvehjem (1933) using this reagent developed a colorimetric method sensitive to 0·01 mg. of cobalt. They did not remove iron and copper but trusted to the action of nitric acid to destroy the colour due to these metals. Kidson and others (1936) improved the method until they were able to detect 0·0001 mg. They found that copper, even in small amounts, interferes to some extent with the determination of cobalt and must be removed as sulphide. Iron must also be removed, preferably by ether extraction of the chloride since if precipitated with ammonia it is liable to adsorb cobalt. Chromium and manganese also interfere. McNaught (1939, 1942) by further modifications of the method was able to determine as little as 0·00005 mg. of cobalt. The organic matter of the sample is first destroyed by wet oxidation with nitric and sulphuric acids and the acids driven off by heat. The residue is heated for a few minutes to 500° and dissolved in hydrochloric acid, iron is removed by extraction with ether, and copper by precipitation with hydrogen sulphide. The residual cobalt is oxidised with nitric acid and the colorimetric determination with nitroso-R-salt carried out in dilute nitric acid solution. For details of the method and the precautions to be taken to secure the highest degree of sensitiveness, McNaught's papers should be consulted.

Macpherson and Stewart (1938) recommend the use of a Zeiss photometer in Stare and Elvehjem's method. By using appropriate colour screens, supplied with the instrument, the colours given by nitroso-R-salt with iron and copper can be eliminated, and their removal from the solution, when they are present in small amounts, can be dispensed with. When larger amounts of these metals are present it is preferable to remove them, copper by precipitation with hydrogen sulphide or with metallic aluminium and iron by precipitation with zinc oxide. Lugg and Josland (1936) found that Stare and Elvehjem's method failed when large amounts of calcium were present. They proposed a modification in which calcium phosphate is kept in solution by the addition of citrate.

Sylvester and Lampitt (1940) proceed on rather different lines, making use first of nitroso-β-naphthol to precipitate iron, copper and cobalt together and subsequently separating cobalt by extraction with diphenyl-thiocarbazone (dithizone) before determining it colorimetrically with nitroso-R-salt. The material is ashed at a low heat in a silica, or preferably platinum, dish with small amounts of sulphuric and nitric acids, the ash is taken up with hydrochloric acid, and iron, copper and cobalt precipitated with a 1 per cent solution of α-nitroso-β-naphthol in glacial acetic acid. The liquid is heated and allowed to stand overnight at room temperature. After filtration the precipitate is heated with sulphuric and perchloric acids to destroy organic matter. The acid liquid remaining after oxides of chlorine have been removed by boiling is diluted with water, and any copper present extracted by repeated shaking with a solution of dithizone in chloroform (cf. p. 48), cobalt and iron remaining in solution. The residual liquid is made just alkaline with ammonium citrate and ammonia, and cobalt extracted with dithizone in chloroform, iron remaining in solution. This extract is evaporated to dryness, organic matter completely oxidised with sulphuric and perchloric acids, and the colourless residue used for the determination of cobalt.

For this it is treated with about 6 ml. of water and made slightly alkaline to phenol-phthalein with sodium hydroxide solution. Hydrochloric acid is added until the liquid is just acid and then 1 g. of sodium acetate followed by 2 ml. of a 0·2 per cent aqueous solution of nitroso-R-salt. The liquid is boiled gently for 2 minutes, 1 ml. of nitric acid is added and the mixture again boiled for 1 minute. After cooling, it is made up with water to 10 ml. and filtered clear through a close-grained filter-paper (Whatman No. 42). The colour of the solution is then measured in a Lovibond tintometer, or the optical density may be determined in a Zeiss photometer and the cobalt content obtained from a graph. Alternatively the colour of the solution may, of course, be compared with that of a series of standards containing known amounts of cobalt. The method is sensitive to 0·005 p.p.m. of cobalt in foods such as milk and fruit.

Sylvester and Lampitt also suggest a qualitative confirmatory test for use when the amount of cobalt present is extremely small. The test makes use of the principle of chromatographic analysis, the red cobalt compound being adsorbed on alumina containing the merest trace of a vegetable oil or fatty acid. A positive result is obtained with as little as 0·0002 mg. of cobalt.

WITH POTASSIUM DITHIO-OXALATE. Cobalt gives a brown colour with potassium dithio-oxalate, stable in presence of potassium cyanide. The method is described under nickel (p. 280).

POLAROGRAPHIC METHOD. Cobalt can be determined by the polarograph (p. 53), but if zinc is present in any quantity it must first be removed by extraction with dithizone at pH 4·0 to 4·5. Iron also interferes with the determination and must be removed (Heller and others 1937).

SPECTROGRAPHIC METHODS. Scott and Mitchell (1943) have adapted the spectrographic method which they used for the determination of chromium in soils (p. 443) to that of cobalt, nickel and molybdenum in soils and pastures. They find that to get accurate results it is necessary to concentrate these metals by precipitation with 8-hydroxyquinoline at a pH of about 5·2. The precipitate is ashed and the resulting ash, diluted with silica powder, is examined with a spectrograph using the cathode layer arc method.

Iron is used as an internal standard, the amount present being checked by Scott's salicylic acid colorimetric method (p. 266). An accuracy of ± 10 per cent is claimed in determinations of cobalt, nickel and molybdenum.

If a preliminary chemical separation of these elements is necessary it may perhaps be questioned whether the spectrographic method is preferable to the extremely delicate colorimetric methods available for cobalt and nickel, but the spectrographic method undoubtedly has the advantage that it is absolutely specific and also that it is possible to determine several trace elements simultaneously on the same photographic plate. Scott and Mitchell give a bibliography of spectrographic methods of determining trace elements in which preliminary chemical concentration has been found necessary.

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11

[MANGANESE]

MANGANESE seems to be essential to plant and animal life although no actual compounds of it have been identified in tissues. The metal, as is well known, forms at least six different oxides, and its rôle in nature may be that of an oxidation catalyst.

FUNCTION OF MANGANESE IN PLANTS. Manganese is located mainly in the leaves and actively growing parts of plants and appears to be connected in some way with the synthesis of chlorophyll (Bertrand and Rosenblatt 1932, McHargue 1924). It probably operates in maintaining the proper oxidation-reduction potential in the plant tissues to enable iron to take part in the synthesis of chlorophyll (Sherman 1944) and for the assimilation of carbon dioxide to form carbohydrate (Gerretsen 1937). Etiolated leaves contain less manganese than green leaves. Wain, Hunt and Marsh (1939) found that in meadow grass manganese was at its lowest in spring (50 to 100 p.p.m. in the dry matter according to species) and highest in December in the dead and dying grass (170 to 250 p.p.m. in the dry matter). This seems to indicate that manganese is continually being absorbed and accumulated during the growing season and reaches its highest concentration in the latest stages of growth.

EFFECT OF MANGANESE DEFICIENCY IN SOIL. Lack of available manganese produces chlorosis in plants. In particular it is responsible for ‘ grey speck ’ of oats, a disease characterised by withering and greying of the tips of leaves and shoots, ‘ speckled yellows ’ of sugar-beet leaves, and ‘ marsh spot ’ of peas, in which the cells of the seed die and a brown spot appears on the flat surfaces of the two cotyledons. Coloured illustrations of some of these conditions have been given by Wallace (1943, 1944). Gerretsen (1937) suggests that grey speck of oats is caused primarily by soil bacteria and that a high manganese content keeps the plant healthy and enables it to resist invasion by bacteria. According to Leeper (1941) oats suffering from manganese deficiency give an intense reaction for nitrate. Apparently manganese is essential to the plant to enable it to assimilate nitrates taken up from the soil (Burström 1939), and in its absence nitrates tend to accumulate in the plant tissues. De Gregorio in 1916 claimed that manganese was required for the fixation of nitrogen in soil by *Clostridium pasteurianum* and *Azotobacter chroöcoccum.* The optimum concentration in artificial media was 60 p.p.m. of soluble manganese. In general, leguminous plants are more sensitive than non-leguminous to lack of manganese. Peas suffering from marsh spot contain less than 5 p.p.m. of manganese instead of the 15 p.p.m. found in healthy peas (Glasscock and Wain 1940).

Seeds normally contain from 20 to 60 p.p.m. of manganese (Wester 1921), which is enough for 6 to 8 weeks' growth on manganese-free soils, and deficiency symptoms do not therefore appear in the early stages of growth (McHargue 1922).

Wain (1938) found that a number of soils on farms of the South Eastern Agricultural College at Wye contained from 260 to 780 parts of total manganese per million, but that because of their alkalinity, pH 7·0 to 8·4, only 0·2 to 1 p.p.m. was available as measured by solubility in normal ammonium acetate solution. This small amount of available manganese was considered to be near the minimum requirement for most plants, and ‘ marsh spot ’ of peas had been recorded as occurring in the area in question. On a neighbouring brick earth soil of pH 5·45 the total manganese was 290 p.p.m. and the available 6 p.p.m. Grass grown on the alkaline soils contained 50 to 110 p.p.m. of manganese in the dry material as against 430 p.p.m. in grass from the brick earth soil. Leeper (1934) considers that manganese deficiency is caused not so much by lack of ammonium acetate soluble, immediately available manganese which he puts at less than 1 p.p.m. for all fertile soils, as by too low a content of 'active MnO_2' or readily reducible manganese oxide. He measures this by extraction with a 0·2 per cent solution of quinol in normal ammonium acetate at room temperature, the manganese oxidising the quinol and going into solution. The active manganese dioxide usually amounts to more than 100 p.p.m. of the soil. If it is less than 15 p.p.m. the soil is deficient and it would be dangerous to lime it as this would cause still further loss of active manganese (cf. also Sherman, McHargue and Hodgkiss 1942). Twyman (1944) gives analyses of various soils in terms of (*a*) water-soluble, (*b*) exchangeable (neutral ammonium acetate-soluble) and (*c*), easily reducible (quinol-soluble) manganese, and confirms Leeper's and Sherman's conclusion that the easily reducible manganese is the important fraction. Most soils contain ample manganese in this form. Deficiency when it occurs is confined to soils of pH 6·7 or more, especially heavily limed soils containing much organic matter (*Rothamsted Exp. Sta. Ann. Rept.* 1938, Wallace 1940).

Mann and Quastel (1946) find that manganese undergoes a metabolic cycle in soils, the kinetics of which are determined by the nature of the micro-organisms and the organic matter present. Bivalent manganese is oxidised by soil bacteria to Mn^{III} and Mn^{IV} which, in presence of substances which can act as hydrogen donators, are reduced to Mn^{II} by other micro-organisms. The relative proportions of the higher and lower oxides in any soil are therefore dependent on the nature of the soil and its bacterial flora.

Several investigators have found, in pot culture experiments, that there is an optimum ratio between iron and manganese in soils. This ratio appears to be about 2 parts of iron to 1 part of manganese. If excess of either metal is present, symptoms indicating deficiency of the other metal appear. Plants will grow in soils with widely different levels of manganese and iron provided that the correct balance between the two is maintained. It is suggested that in the absence of manganese, ferrous iron may exist in toxic amounts, but if manganese is present iron is oxidised to the ferric state and becomes inactive. With excess of manganese all the iron is oxidised, and not enough ferrous iron remains for the needs of the plant (Pearse 1944).

Manganese deficiency can be remedied in many instances by the application of 15 to 40 lb. of manganous sulphate per acre, which would correspond roughly to 2–4 p.p.m. of manganese in the top 12 inches of soil. Fruit trees can be treated by spraying with manganese sulphate and by insertion of manganese salts in holes in the trunk of the tree (Epstein and Lilleland 1942).

TOXICITY OF MANGANESE IN SOILS. Although essential in small quantities, manganese is toxic to plants when present in excess. Its toxicity, like that of iron, depends upon the amount of directly soluble manganese in the soil. In water culture, very small concentrations, of the order of 0·5 to 1 p.p.m., may be toxic (Olsen 1936), but in soil the toxic limit for soluble manganese is probably higher. Wallace and others (1945), in sand culture experiments with nutrient solutions at pH 5·2, found that manganese was particularly toxic at low levels of calcium. Thus in acid soils containing little or no lime, plants are more likely to suffer from manganese poisoning than in alkaline or neutral soils. Plants which dislike lime and which grow best in acid soils are often tolerant of manganese. The ash of spruce wood, for instance, may contain as much as 22 per cent (Adamek 1937): water plants also contain relatively large amounts.

The suggestion that manganese poisoning of plants may sometimes be due to removal of all ferrous iron by oxidation has been referred to above.

MANGANESE IN THE HUMAN AND ANIMAL ORGANISM. Human and animal tissues and organs range in manganese content from 0·2 to 4 p.p.m., the largest amounts being found in the liver and pancreas and in the hair (McCrae 1923). Human liver contains about 1·7 p.p.m., or 7 p.p.m. in the dry material (Brückmann and Zondek 1939), and muscular tissue 0·17 p.p.m.; the contents of other organs average from 0·3 to 0·4 p.p.m. Human blood contains about 0·15 p.p.m. (Kehoe and others 1940). In domestic animals the amounts are similar, although somewhat higher in certain organs. Fish livers are very low in manganese compared with the livers of land animals. It is noteworthy that animal tissues are in general poorer in manganese than plant tissues, whereas with iron the reverse is the case.

There is one species of shell-fish, *Pinna squamosa*, the blood pigment of which (pinna-globulin) contains manganese in place of iron or copper, but so far as is known at present this is an isolated experiment on the part of nature (Pryde), comparable with the occurrence of vanadium in the blood pigment of certain marine worms (p. 477).

The effect of manganese on animals in general is similar to that on plants. In very small quantities it is clearly essential but in large amounts it may be toxic. Pigs grow well on food containing 0·5 p.p.m. of manganese but reproduction and lactation are faulty. 6 p.p.m. is sufficient for normal reproduction (Johnson 1943). Rabbits die if kept on a synthetic diet containing no manganese (Rudra 1944). With male rats its presence in food is necessary to reproduction ; with females lack of it in the food causes the young rats to be weakly with a high mortality rate. The storage of manganese during foetal development seems to be of great importance to the subsequent growth of the young. It is stated that hen's eggs do not hatch well if there is too little manganese in the food. Perla and Sandberg (1939) claim to have found a relationship between manganese and vitamin B_1 in rats ; in fact Perla suggests that without manganese vitamin B_1 is toxic. An important contribution has been made by Edlbacher and Pinösch (1937), who find that nearly all the manganese in the liver of domestic animals (2 to 4 p.p.m. in the moist tissue), and also in yeast, is associated with the enzyme arginase, and that arginase inactivated by dialysis can be re-activated by manganese. They suggest that arginase may consist of a colloidal carrier of albumin type with an active prosthetic group or co-enzyme in which manganese probably plays a part. Arginase hydrolyses arginine to urea and ornithine, a diamino-valerianic acid.

Various other activities have been attributed to manganese by different workers, such as stimulation of tissue respiration, synthesis of ascorbic acid in plants, activation of phosphatases, peroxidases, intestinal peptidases and other enzymes and stimulation of growth of lactic acid bacteria. The last-named action is made use of in the preparation of media for the biological determination of members of the vitamin B complex using lactic acid bacteria. The suggestion that manganese may be concerned in the formation of haemoglobin has not been confirmed.

ABSORPTION, EXCRETION AND TOXICITY OF MANGANESE. Manganese dioxide is not readily absorbed from the alimentary tract and is comparatively harmless in large doses, but its inhalation in the form of dust and eventual entrance into the circulation from the lungs is a well-known industrial risk and leads to degenerative changes in the liver, and at a later stage effects upon the central nervous system. Soluble bivalent manganese salts may be toxic when ingested. Among animals, rabbits seem to be specially susceptible to manganese poisoning ; 0·2 to 0·3 g. of bivalent manganese daily for 3 weeks interferes with bone growth and eventually causes toxic symptoms with damage to the liver (Carratalá and Carboneschi 1935, Kanimura 1938). Richards (1930) fed 3·5 g. of manganese citrate daily to pigs for 9 months without any toxic symptoms appearing, and Skinner (1932) found that 1,000 to 2,000 p.p.m. (0·1 to 0·2 per cent) of manganese in the food of rats did not affect their growth rate. Larger amounts have been found to interfere with phosphorus retention and to promote excretion of phosphate. On diets exceptionally high in phosphorus and calcium, e.g. dried milk, rats have been given 0·1 g. of manganese daily without any apparent injury (Becker and McCollum 1938). Hens can take 1,000 p.p.m. or more in their food, corresponding to about 0·25 g. daily, without ill effects (Gallup and Norris 1939), but 4,800 p.p.m. (0·48 per cent) is highly toxic to young chicks (Insko and others 1938). These figures all refer to soluble manganous compounds and point to a figure of roughly 0·1 g. per lb. body weight as the critical limit of intake. Salts of permanganic acid are far more toxic, showing in this respect a close analogy with chromates ; 0·12 g. of potassium permanganate is stated to produce symptoms of poisoning in man.

Grass grown on certain soils and particularly rich in manganese (540 to 1,320 p.p.m. in the dry matter as against 50 to 100 p.p.m. for normal grass) has been held to be associated with a temporary lowering of the magnesium content of the blood and to lead to lactation tetany in cattle and sheep (Blakemore and others 1937), but Green (1944) considers that the alleged toxicity of pastures containing a high manganese content has never been confirmed.

The absorption of manganese from food resembles that of iron ; normally it is very small. After dosage with fairly large quantities there is a temporary slight increase of manganese in the blood (Reiman and Minot 1921). It appears to be eliminated chiefly in the bile and also through the intestinal walls, only the merest trace appearing in the urine. Kent and McCance (1941) found that even on high manganese diets averaging 8 to 9 mg. daily the urinary excretion was less than 0·1 mg.

PEROSIS IN CHICKS. Perosis or ‘ slipped tendon ’ of chicks is characterised by gross enlargement and malformation of the tibio-metatarsal joint with displacement of the tendon, also by arrested growth of leg and wing bones and of the spinal column (Caskey and others 1939). It appears to be due to manganese deficiency and to be aggravated by high intakes of calcium phosphate (3 to 5 per cent in the food) and also by ferric hydroxide. The higher the calcium and phosphorus content of poultry food the more manganese is required to prevent the occurrence of perosis. According to Schaible and Bandemer (1942) 3 p.p.m. of manganese soluble in 0·1N hydrochloric acid in the undried ration is enough to prevent perosis if the calcium and phosphorus content are normal. The average total manganese content of feeding stuffs is given as 66 p.p.m. in the dry matter (Elvehjem and Hart 1928).

Rice bran, which has a high manganese content (350 p.p.m.), will prevent perosis, but if it is previously heated in an autoclave the active factor is destroyed (Wiese and others 1938), which indicates either that the manganese is thus made insoluble and inactive, owing possibly to its removal from solution by calcium phosphate (Wilgus and Patton 1939), or that other factors besides manganese deficiency are involved. According to Jukes (1941) choline is associated with manganese in preventing perosis, and administration of manganese is not effective if choline is deficient in the food. Cereal grains alone cannot supply more than one-third of the choline required, and animal products must be added as a source of additional choline (Rhian 1941). Betaine can be partially substituted for choline (McGinnis and others 1942). Nicotinic acid deficiency may also be a contributory factor (Briggs 1946).

Coombs, Norris and Heuser (1942) consider that manganese deficiency is accompanied by a lowering of the bone phosphatase level, with consequent retardation of bone development. This is particularly marked when vitamin D is also deficient. According to Miller and others (1940) pigs develop bone affections on diets deficient in manganese and are cured by the addition of manganese sulphate to the diet. Rabbits are similarly affected. The action of manganese on bone development in animals suggests that it may also be connected in some way with the growth of bone in the human body. Kehoe, Cholak and Story (1940) found from 1·7 to 3 p.p.m. in human bone.

MANGANESE IN FOODS. The distribution of manganese in some common foods is roughly as follows, in parts per million of the fresh foods.

Nil to 0·5 p.p.m.—beef, mutton, veal, bacon, poultry, eggs, milk (0·04 p.p.m.), milk powder, butter, salmon, codfish, halibut, mackerel, crab, lobster, prawns, shrimps, cockles, cod-liver oil, olive oil, honey (light), mustard, coffee, lemons, leeks.

0·5 to 2 p.p.m.—kidney, pork, cheese, egg yolk, cabbage, cauliflower, carrots, cucumber, endive, vegetable marrow, asparagus, turnips, mushrooms, potatoes, tomato, rhubarb, radish, cornflour, arrowroot, olives, dates, plums, wine, mussels.

2 to 10 p.p.m.—liver, artichokes, beets, runner beans, broccoli, brussels sprouts, onions, green peas, water-cress, parsley, white bread and flour, rye flour, tapioca, cranberries, blackberries, currants, bananas, prunes, figs, honey (dark), brazil nuts, oysters, molasses, pressed yeast.

10 to 30 p.p.m.—spinach, kale, lettuce, dried peas and beans, rice, barley, coconut, raspberries, almonds, chocolate, gelatin.

Above 30 p.p.m.—oatmeal (36 p.p.m.), wholemeal flour (10 to 70), bran (50 to 150), soya bean meal (40), cocoa (35), cloves (450), tea (150 to 900), chestnuts (40), pepper (65), maple sugar (10 to 120), whortleberries (40 to 200), winkles (40 p.p.m.).

Peterson and Skinner (1931) give the following figures as representing the average manganese content of different classes of food.

	p.p.m.
Nuts	22·7
Cereals and their products	20·2
Dried legume seeds	20·0
Green leafy vegetables	4·5
Dried fruits	3·3
Roots, tubers and stalks	2·1
Fresh fruits (excl. whortleberries)	2·0
Non-leafy vegetables	1·5
Animal tissue	1·0
Poultry and poultry products	0·5
Dairy products	0·47
Fish and sea foods (excl. oysters)	0·25

Boycott and Cameron (1930) also have given a number of figures for the manganese content of foods.

Among spices, cloves are particularly rich in manganese (450 p.p.m.). Tea also contains from 150 to 900 p.p.m. and Kenya tea as much as 1,380 p.p.m. (Coleman and Gilbert 1939). The amount in the infusion as ordinarily drunk may range from 1 to 7 p.p.m., representing roughly one-third of the total manganese in the dry tea.

Among fruits, raspberries and whortleberries are high in manganese. Whortleberry ash is stated to contain 2 per cent and that of raspberries to average 0·5 per cent. Peterson and Lindow (1928) found great differences in the manganese content of similar plants, some containing two or three times as much as others of the same species. These findings could not be correlated with any other factor and were probably due to fluctuations in the amount of available manganese in the soil. Considerable variation may occur also in plants at different stages of growth, and the figures given above for manganese in foods can therefore be taken only as a rough guide.

In a detailed survey of the food budget in winter of two adults, a married couple, on a weekly income of about £5, carried out in 1937, the daily intake of manganese was calculated to be about 7 mg., nearly half of which was contributed by tea at the rate of 1 litre of an infusion containing 3·3 p.p.m. of manganese. The distribution in the daily food was roughly as follows :

Tea	3·3 mg.
Bread (white and brown) Biscuits (plain and wholemeal)	2·2 mg.
All other foods	1·5 mg.
	7·0 mg.

The amount of manganese derived from tea will of course vary widely according to the kind of tea, the strength of the infusion and the amount drunk, but for many people tea is probably by far the richest source of dietary manganese.

The minimum daily requirement of manganese is not known. Everson and Daniels (1934) suggest that children of 3 to 5 years should receive 0·2 to 0·3 mg. per kilogram of body weight, which would amount to about 3·0 to 5·0 mg. daily for a child of 35 lb. This estimate seems rather high and would barely be covered by an ordinary child's diet. Basu and Malakar (1940) give 4·6 mg. daily as the amount required to keep an adult male in manganese balance. There is no reason to suppose, on the information at present available, that manganese deficiency ever occurs in human beings, but comparatively little work has been done on the subject. It would seem that the manganese requirements of domestic animals, especially poultry, may be considerably higher than those of human beings, but this requirement is normally satisfied by the higher manganese content of animal feeding stuffs.

Manganese in water has an effect similar to that of iron. It imparts a taste to the water and also favours the growth of iron bacteria (p. 248). Large amounts, of the order of 300 p.p.m., are stated to be toxic to fish.

THE DETERMINATION OF MANGANESE IN FOOD

Manganese in food and biological material is usually determined by oxidation to permanganate with potassium or ammonium persulphate or with potassium periodate. The pink permanganate solution is then compared with standards. Sodium bismuthate and lead peroxide have also been used as oxidising agents.

OXIDATION WITH PERSULPHATE. This method was introduced by Marshall in 1901. The ash of the sample is heated with potassium or ammonium persulphate in presence of sulphuric or nitric acid and a small quantity of silver nitrate which acts as a catalyst. A distinct pink colour is given by 0·001 mg. of manganese in 0·5 ml. of solution. Marshall considered that silver peroxide is first formed in the reäction mixture and that this is the active agent in oxidising manganese salts to permanganate.

Chlorides interfere with the reaction, presumably by precipitating silver chloride and preventing the formation of silver peroxide, and must be removed by heating the ash with nitric or sulphuric acid (Davidson and Capen 1931). If much calcium is present sulphuric acid causes the precipitation of calcium sulphate, which adsorbs manganese and leads to low results (Mühlenbach 1938). It is preferable, therefore, with materials rich in calcium, to carry out the oxidation with the minimum amount of persulphate in dilute nitric acid. Ray (1940) removes chlorides by evaporating a nitric acid solution of the ash with a very small quantity of sulphuric acid, insufficient to precipitate calcium sulphate. Silica, if present in appreciable amount, may also adsorb manganese salts, and titanium in amounts exceeding 1 per cent may interfere with the development of the permanganate colour (Hough 1935).

If much iron is present, phosphoric acid should be used instead of sulphuric. This, by de-ionising iron, removes the yellow colour due to ferric salts and also prevents the precipitation of hydrated manganese dioxide from substances rich in manganese (Alten and Wieland 1933).

Marshall (1901) recommended that dilute standard permanganate solutions should be reduced before being stored for any length of time, and re-oxidised to permanganate before use. Otherwise they are liable to lose strength by deposition of manganese oxides on the surface of the glass. Richards (1930) also reduces standard permanganate solution with sulphur dioxide and oxidises it to permanganate with periodate before use. Dilute permanganate solution thus oxidised and containing a slight excess of periodate is remarkably stable.

Cheramy and Lemos (1937) treat biological material by wet oxidation, and after heating with persulphate and silver nitrate they titrate the resulting permanganate with standard hydrogen peroxide solution. This method would seem to be suitable only for comparatively large amounts of manganese.

OXIDATION WITH PERIODATE. The advantage of periodate over persulphate as an oxidising agent is that silver nitrate is not required as a catalyst, and traces of chlorides do not therefore interfere. It is, however, advisable to remove them by heating with sulphuric or nitric acid, otherwise there is a danger that the colour will fade owing to the action of hydrochloric acid upon permanganate (Richards 1930).

It is important to control the acidity of the solution in which the oxidation is carried out. If the solution of the ash is too acid (more than 15 per cent by volume of sulphuric acid) the colour of the permanganate fades quickly to a yellowish tint. Fading also takes place if the acidity is below 1 per cent. Richards recommends an acidity of 5 to 6 per cent of sulphuric acid by volume. The amount of free acid must be enough to prevent precipitation of manganese periodates or oxides. If much iron is present phosphoric acid should be substituted for part of the sulphuric acid, or pyrophosphate may be added, which is more efficient in de-ionising iron (Wain 1938). With materials such as straw containing much silica, which may adsorb manganese salts, silica must be removed by heating with hydrofluoric acid in a platinum dish (Davidson 1931).

In Smith and Deszyck's method (1940) for determining manganese in grain and stock feeds, suggested for official adoption by the Association of Official Agricultural Chemists, the solution in which the oxidation is carried out contains 2·5 ml. of sulphuric acid and 12·5 ml. of approximately 8 per cent phosphoric acid in 40 ml., and the oxidation is effected with 0·3 g. of potassium periodate. The method is sensitive to about 0·007 mg. of manganese. Richards (1930) uses a similar concentration of acid but a much smaller volume of solution (0·5 ml. H_2SO_4, 3 ml. of 8 per cent H_3PO_4 and 0·3 g. of KIO_4 in 10 ml. of solution), and this increases the sensitiveness of the method to 0·002 mg. of manganese.

Silica dishes are not very suitable for direct ashing of foods owing to the action of alkaline ash on the surface of the silica. It is better to work with a sulphated ash. With materials such as milk, containing large amounts of calcium, Richards removes most of the calcium as sulphate by treatment of the ash with sulphuric acid of 33 per cent strength by volume. The calcium sulphate precipitate is filtered and washed several times with the 33 per cent acid, a slow operation. It is preferable to allow calcium sulphate to settle and to work on an aliquot part of the clear liquid.

The following adaptation of Richards' method gives excellent results with quantities of manganese down to 0·002 mg. If the sample is small, organic matter is destroyed by wet oxidation with about 5 to 6 ml. of sulphuric acid and nitric acid as required. If the sample is large, or contains much fat, it is charred in a silica dish and the char moistened with about 2 ml. of sulphuric acid. Ashing is then completed at a dull red heat, using if necessary a little nitric acid. The ash is treated with 5 ml. of sulphuric acid and a little water, and the solution heated until the sulphuric acid fumes, cooled and diluted. The acid solution is washed into a 25-ml. graduated flask, cooled again and made up to volume. If the solution is turbid it is allowed to remain overnight for calcium sulphate to settle. It is filtered through a small No. 44 Whatman filter-paper and 20 ml. (i.e. four-fifths) evaporated to dryness in a silica dish, sulphuric acid being fumed off. The residue is dissolved in 0·5 ml. of sulphuric acid, 3 ml. of approximately 8 per cent phosphoric acid solution and about 3 ml. of water added, and the solution heated if necessary to clear it. It is then washed into a test-tube with small quantities of water until the volume measures 10 ml. 0·3 g. of potassium periodate is added, the tube covered with a glass cap, the contents heated to boiling and the tube placed in a boiling water bath for 30 minutes. A control experiment is carried out at the same time on the reagents. The solutions are cooled and transferred to small flat-bottomed specimen tubes, and the permanganate colour is matched by adding to the control a standard solution of potassium permanganate (1 ml. = 0·01 mg. of manganese).

With large amounts of manganese excess of periodate can be removed by precipitation as mercuric periodate, and the permanganate in the filtrate determined in the ordinary way with ferrous sulphate (Willard and Thompson 1931, Read 1939).

Balin (1934) recommends fusing the ash of plant material with sodium carbonate, and claims to get a higher recovery of manganese than by the ordinary method. Manganese in the ash is reduced with sulphur dioxide and afterwards oxidised to permanganate with periodate.

OXIDATION WITH SODIUM BISMUTHATE. Sodium bismuthate, $NaBiO_3$, is a yellow or brown insoluble amorphous powder prepared by passing chlorine into a suspension of bismuth trioxide or hydroxide in sodium hydroxide solution. It was used by Collins and Foster (1924) for the determination of manganese in water. 100 ml. of the water, containing not more than 1 mg. of manganese, are evaporated with dilute (1 : 3) nitric acid (10 ml.) and concentrated sulphuric acid (1 ml.), and the residue heated to expel nearly all the sulphuric acid. It is then dissolved in dilute nitric acid (free from lower oxides of nitrogen), 0·1 g. of sodium bismuthate is added, the liquid is stirred for one minute and filtered through asbestos and the colour compared with that of a standard permanganate solution containing the same amount of nitric acid.

According to Davidson and Capen (1931) the bismuthate method gives slightly low results. One objection to it is that no excess of oxidising agent remains in the liquid to stabilise the colour of the permanganate.

BENZIDINE METHOD. If permanganate is allowed to act upon benzidine in dilute acetic acid solution, a blue oxidation product is produced, and this furnishes a more delicate reaction for manganese than the colour of permanganate itself. Clark (1933) found that the blue colour could be stabilised with small amounts of alkali nitrates or chlorides, but that with very low concentrations it was apt to fade. It was possible to detect 0·001 mg. of manganese in 50 ml. Wiese and Johnson (1939) used benzidine in conjunction with oxidation by sodium bismuthate. They found the yellowish-green colour of oxidised benzidine in dilute nitric acid to be more stable than the blue colour in acetic acid. The ash of the sample is dissolved in dilute nitric acid and evaporated to dryness twice to remove chlorides. The residue is taken up with dilute nitric acid, oxidised with sodium bismuthate, the excess of bismuthate filtered off and the filtrate treated with a small quantity of benzidine in dilute acetic acid. The yellowish-green colour which develops is compared with standards.

OXIDATION WITH LEAD PEROXIDE. Manganese salts may be oxidised to permanganate by boiling with lead peroxide and nitric acid. According to Iyengar (1938) this method is more accurate and more sensitive than either the bismuthate or the persulphate methods. Solarino (1928) dissolves the ash from 100 ml. of wine in concentrated nitric acid, dilutes with water and boils the solution with about 0·5 g. of lead peroxide. The permanganate formed is titrated with 0·01N oxalic acid, 1 ml. of which is equivalent to 0·00011 g. of manganese. This method was found to be suitable for wines containing from 1 to 3 p.p.m. of manganese.

For the colorimetric determination of very small amounts of manganese the same objection applies here as with the bismuthate method, that no excess of oxidising agent remains in solution to stabilise the colour of the permanganate.

FORMALDOXIME METHOD. A solution of formaldoxime, H.CH : N.OH, is made by heating 3 g. of trioxymethylene, $(CH_2O)_3$, with 7 g. of hydroxylamine hydrochloride and 15 ml. of water until the liquid is clear. Alternatively it may be prepared by adding formalin to a solution of hydroxylamine without heating. It gives colours with copper (blue-violet), cobalt (straw yellow), nickel (brown), manganese (orange-brown) and iron (brown-violet). The reaction with manganese is the most sensitive, and it is possible to detect 0·08 p.p.m. in solution (Wagenaar 1938). Copper, cobalt and nickel can be completely, and iron partially, immobilised by adding cyanide, but iron still gives a greenish-yellow colour in presence of cyanide and must therefore be added in equal amount to the standard solution so as to compensate for the colour (Sideris 1937, 1940). Iron may also be removed by oxidising it to the ferric state and precipitating it with ammonium oxalate or zinc oxide (Wagenaar 1938, Kahane 1935). Phosphates interfere with the reaction and can be removed by precipitation with lead acetate in acetic acid. The sample is ashed, the ash dissolved in dilute hydrochloric acid and the solution neutralised and acidified with acetic acid. If much phosphate is present it is precipitated with lead acetate and excess of lead removed with sodium sulphate. To the filtrate are added sodium hydroxide and formaldoxime solution and the colour compared with standards (Sideris 1940).

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12

[IODINE]

IODINE has received more attention than any other trace element in nutrition owing to its importance as an essential constituent of the thyroid gland, the secretions of which are indispensable to growth. A deficiency of iodine in early life results in a diseased and often immensely swollen gland at the base of the neck, and in severe cases cessation of growth and cretinism.

The iodine question has been so thoroughly dealt with in numerous books and reports that only a short general survey will be given here of some of the points of special interest and importance to the food chemist. Fuller information is to be found in reports by Orr and Leitch (1929), Orr (1931), Young, Crabtree and Mason (1936) and McClendon (1939).

DISTRIBUTION OF IODINE IN NATURE. The quantities of iodine occurring in natural products are so small that it is usual to refer to them in terms of micrograms (μg. or γ) per kilogram, that is, in parts per 1,000 million instead of parts per million.

Iodine in Soil and Water. It is popularly supposed that the chief source of iodine is the sea and that iodine deficiency, as shown by the prevalence of endemic goitre, is largely confined to mountainous districts remote from the sea. Goitre is certainly endemic in many mountainous regions, but as far as this is due to lack of iodine it is apparently rainfall and soil conditions which are responsible rather than distance from the sea. Numerous analyses have shown that the rocks of the earth's crust, and the soils derived from them, are the chief repository of iodine and that the intake in food depends very little, if at all, on distance from the sea. Sea-water itself contains 17 to 18 μg. per litre according to Orr and Leitch, or 50 μg. according to Goldschmidt (1937), which are relatively small amounts. It is true that seaweed and plankton are rich in iodine absorbed from sea-water, and that the air on the sea-shore contains iodine given off from seaweed (Dangeard 1928) and in the plankton thrown up with spray, but beyond a narrow margin on the coast the effect of this on the iodine content of soil and crops is negligible (Remington and others 1929). In the few instances in which it has been possible to find a definite correlation between iodine distribution in nature and the incidence of goitre, it is the soil iodine which has been found to be significant (Shore and Andrew 1934). Lack of sea fish in the diet, owing to difficulties of supply, may be a contributory factor.

In general the iodine in soils varies from 600 to 8,000 μg. per kilogram. Very little distinction can be drawn between soils of different geological formations in respect of iodine content. Humus and colloid constituents, such as clay, retain iodine. Soils nearly always contain more than the rocks from which they are derived, owing to absorption by plants and retention by humus. Peat soils contain the most iodine (Köhler 1929). Calcareous and sandy soils with low humus and colloid content tend to lose iodine, apparently by leaching. It may, perhaps, also be lost from some soils through oxidation by iron and manganese salts (von Fellenberg and others 1924) and by nitrous acid. Artificial manures (phosphates, Chili saltpetre, etc.) contribute iodine to the soil. Natural rock phosphate is relatively high in iodine (Hill and Jacob 1933).

Fresh water contains comparatively little iodine ; Orr, Godden and Dundas (1928) found 0·05 to 4·5 μg. per litre in twenty water supplies in England and Scotland. Some deep-well waters contain much more, up to 1,200 μg. per litre at Cheltenham and 40,000 μg. per litre in some Continental springs (cf. M.R.C., Memo. No. 18, 1948).

The iodine content of the atmosphere is very small, and varies greatly from place to place. It is usually of the order of 1 μg. per cubic metre and higher over the sea. It may be increased considerably by coal smoke ; as much as

38,900 μg. per kilogram has been found in soot (Orr and Leitch 1929). Dust-free air contains no iodine ; it exists in the bodies of micro-organisms and in dust particles and is largely removed from the air by rain. Free iodine has, it seems, only a transitory existence in nature. Owing to its great activity, when liberated from iodides by oxidation, it soon recombines with any organic matter with which it comes into contact—humus in soils, dissolved or suspended matter in water and organic dust in the atmosphere.

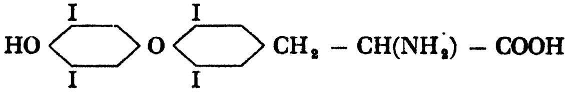
Iodine in Plants. All plants contain iodine, from traces of the order of 2 μg. up to 500 or even 1,000 μg. per kilogram. Seaweeds contain very much more, up to 0·2 per cent in the fresh material. The outstanding feature of the iodine content of plants is the wide variation found in the same species when grown in different districts. It seems to depend more upon the available iodine in the soil than upon the species of plant, although species certainly has some influence (Balks 1936, Fischer 1931, Maurer and others 1928). Thus lettuce shows from 50 to 620 μg. per kilogram according to locality, wheat from 1 to 64, nuts from 15 to 200, and water-cress from 190 to 450 μg. per kilogram (Orr and Leitch). The average figure for cereals in different parts of the world may be taken as 25, for vegetables about 30 and for fruit 10 μg. per kilogram. Dressing with artificial manures containing iodine increases the iodine content of the plant (Orr, Kelly and Stuart 1928), but the increases are irregular and generally confined to the stems and leaves (Gaus and Griessbach 1929). Iodine in small quantities has been found to stimulate the growth of plants, but the results are not sufficiently pronounced or uniform to warrant the application of iodides in practice. Sugar beet is perhaps an exception and iodine manuring has in many instances resulted in a heavier crop.

The iodine in vegetation appears to be in organic combination and is not lost on cooking (Miermeister 1929).

Iodine in Other Foods. Meat contains about 30 μg. per kilogram, vegetable oils about 50, milk 10 to 70, eggs 10 to 400 with an average of about 100, fresh-water fish about 40, sea fish about 400 and shell fish 900. These figures can be taken only as rough indications; the variations are mostly so great that average figures are of little significance. Sea-fish oils are extremely rich in iodine, up to several hundred parts per million (Heddle and Brawn 1940).

Iodine in Stock Feeding. In some regions where human goitre is prevalent, animals also may suffer from diseases due to iodine deficiency, characterised by hairlessness and still-births of the young, or subnormal growth, and the administration of iodine has produced good results. Apart from this, iodine has frequently been tried as an addition to animal feeding-stuffs, and in some instances increased rates of growth have been recorded, but the results are too uncertain to have much practical value except in known go itrousregions. It is claimed that feeding of iodinated proteins to dairy cows increases the milk yield to some extent, but in general iodine dosage of cows does not give more than a slight temporary advantage, although it greatly increases the iodine content of the milk. Similarly with poultry it is easy to raise the iodine content of eggs to many times the normal, and in some countries hyper-iodised eggs and iodo-brominated eggs have been marketed as an alternative to iodised salt (D'Ambrosio 1933, Straub 1933, Viollier and Iselin 1935). Orr and Leitch give figures for the daily iodine intake of cows, sheep, pigs and poultry based on the iodine content and quantity of food eaten, but it is uncertain what relation these amounts have to the optimum intake, and the minimum requirements of these animals have not been defined.

Iodine in the Human Body. The action of iodine in preventing goitre was known for well over a century before the discovery of its presence in the thyroid gland by Baumann in 1895. Thyroxin, the active principle of the gland, was isolated by Kendall in 1914. It was synthesised by Harington in 1926 and shown to have the structural formula



It is therefore a derivative of tyrosine (β-parahydroxyphenyl-α-aminopropionic acid). Part of the iodine of the normal thyroid gland is present as diiodotyrosine, a product intermediate between tyrosine and thyroxin.

Although the active principle of the gland is now so well known the metabolism of iodine both in health and disease is by no means clear, and it is not even certain that iodine deficiency is the sole cause of goitre and cretinism. Perhaps one of the reasons for this uncertainty is the difficulty of determining accurately in biological material the extremely small quantities of iodine involved, especially when many routine determinations are required. It has been suggested that susceptibility to goitre may be increased by infectious diseases, by water pollution, by diets containing too much fat or by the organic cyanides and thiocyanates which exist in certain vegetables such as cabbage. Thiocyanates, both organic and inorganic, appear to inhibit the formation of thyroxin, possibly by combining with and immobilising iodine. An interesting suggestion is that made by Kraft (1936), from the results of experiments on the metamorphosis of tadpoles, that iodine and fluorine are antagonistic and that the production or action of thyroxin may be impeded to some extent by fluorides. No correlation has been found, however, between fluorine intake and the incidence of human goitre. The effect of fluorides on enzyme action is referred to on page 355 (cf. M.R.C., Memo. No. 18, 1948).

The normal human thyroid gland contains on an average about 8 mg. of iodine, equivalent to about 60 per cent of the total body iodine, but the amount may vary considerably with different individuals and in different localities. The total storage capacity of the gland is not over 30 mg. (Marine 1923). The iodine content of normal human blood is 30 to 150 μg. per litre, the higher figures occurring in autumn and the lower in spring. It is affected by certain diseases of the thyroid gland, being low in simple goitre and high in toxic goitre, but the actual relationship between blood iodine and thyroid iodine, and between organic and inorganic iodine in the body and its significance, is not clear. It seems that simple goitre is connected with deficient synthesis of di-iodotyrosine and thyroxin, and toxic goitre with over-production of these bodies. The anterior pituitary gland is concerned as well as the thyroid and there is a balanced relationship between these two glands which may be upset by irregularities in the production of thyroxin. The whole adult body contains normally 11 to 13 mg. of iodine (Buchholz 1928, Orr and Leitch 1929) but this again may vary greatly. The average amount excreted daily by the kidneys in goitre-free regions seems to lie between 60 and 120 μg. Excretion also takes place in some degree through the intestinal canal and the skin, the proportions leaving the body by these routes varying with the total intake (Scheffer 1933).

MINIMUM IODINE REQUIREMENTS. Although the biochemistry of the thyroid gland is still somewhat obscure, one thing is perfectly clear—a sufficiency of iodine in early life does assist normal growth and is a prime factor in the prevention of goitre and cretinism.

Many attempts have been made to establish the average amounts of iodine ingested and excreted in goitrous and non-goitrous areas, but the great fluctuations in iodine intake from food, water, etc., and the fact that it may be excreted by so many routes (intestinal canal, skin, lungs and kidneys) make it difficult to obtain concordant figures. More exact knowledge of iodine requirements is given by individual metabolism experiments (McClendon and Hathaway 1923, von Fellenberg 1923, 1926, Scheffer 1933, Cole and Curtis 1935). From the data available it has been calculated that the minimum iodine requirement of a child is 50 μg. daily and of an adult 15 μg. It is customary with other mineral elements (e.g. calcium and phosphorus) to allow a generous margin for contingencies, and the minimum daily requirements for iodine are therefore taken by Orr and Leitch as being three times these quantities, that is, 150 μg. for a child and 45 μg. for an adult. Godden (1939) states that an average daily intake of iodine under 1 μg. per kilogram of body weight is insufficient to prevent goitre, while an average intake approaching 2 μg. per kilogram is sufficient.

IODISED SALT. Iodine as a prophylactic against goitre is usually given in the form of iodised salt. About the middle of last century salt containing 400 p.p.m. was in use in France. Assuming that 10 g. of salt is taken daily, the dose of iodine would thus be 4 mg. (4,000 μg.). According to Mühe (1935) this was found to be too high and the use of this salt was discontinued owing to the occurrence of toxic symptoms in a few cases. In the United States this danger is apparently discounted, and salt containing 200 p.p.m. of iodine was at first used but subsequently reduced to 100 p.p.m. In Canada also 100 p.p.m. is favoured. The Swiss goitre commission on the other hand recommended in 1923 a salt containing only 5 p.p.m. of iodine, which was found to be not quite strong enough for the more goitrous districts and was raised later to 10 p.p.m. This recommendation appears to have been generally followed throughout Europe, although commercial samples of the salt have been found to differ widely in iodine content (Prange 1933). 10 p.p.m. implies a daily dose of about 100 μg. of iodine, assuming a salt intake of 10 g. This was held to be sufficient to supplement the natural iodine intake in goitrous districts and bring it up to the optimum intake for children. The success of this treatment was shown by the virtual disappearance of goitre and cretinism in those districts where iodised salt (' voll-salz ') was introduced. In New Zealand the iodine content of iodised salt is fixed by regulations under the Sale of Food and Drugs Act. For some time the standard was not less than 4 and not more than 8 p.p.m. expressed as potassium iodide, but it has since been raised to 37·5–75 p.p.m.

A certain amount of criticism has been directed at the use of ' voll-salz ' by adults. It is pointed out that the thyroid gland is primarily of importance in childhood during the period of growth and that iodine treatment has not the same value in after life, except during pregnancy with the object of preventing congenital goitre. Children tolerate iodine much better than adults and their iodine requirements are three times as great. It is alleged that adults constantly receiving small doses of iodine are liable to develop toxic symptoms. Mühe (1935) examined the available records and found that there was a marked increase in cases of hyperthyroidism in adults after 'voll-salz' was introduced in 1923, which could only be ascribed to the action of iodine in the salt. She quoted a number of authorities in support of her contention and suggested that hypersensitiveness to iodine is fairly common among adults, at any rate in the goitrous districts of Bavaria. Other authorities have stated, in support of the compulsory use of 'voll-salz', that at the highest estimate not more than 2 per cent of the population show iodine hypersensitiveness. They dismiss as negligible the likelihood of any ill effects resulting from the use of iodised salt, and point to the extremely small daily dose involved compared with the very large doses frequently prescribed in medicine and the free use of iodine in tinctures and liniments, much of which is absorbed into the blood. They claim that any excess of iodide over that required to maintain the saturation level of the thyroid gland behaves like other halide salts and is rapidly excreted, chiefly in the urine. In this connection, however, reference may be made to Ucko's work on bromine excretion (p. 337), which showed that bromides are eliminated very slowly from the body. It may be that occasional massive doses of iodides cannot be considered in the same light as daily small doses continued for many years, and that the habitual use of iodised salt, while beneficial and even essential to children, is not altogether without risk to a certain small proportion of adults. Hypersensitiveness to iodine may be commoner in some districts than in others, but even if it affected only 2 per cent of the population this would seem to be a sufficient reason for objecting to the compulsory iodisation of all household, or even all table salt.

Iodised salt, prepared by simple addition of an alkaline iodide, has been found to lose iodine rather quickly. Salt containing 5 p.p.m. of potassium iodide loses one-third of its iodine in six weeks on storage in cardboard containers, owing entirely to gradual migration of iodine to the cardboard or fabric of the container (Andrew 1938, Andrew and Stace

1945). The rate of loss depends to some extent on atmospheric humidity and may be lowered by adding sodium bicarbonate or other stabiliser or by the use of iodate instead of iodide (Johnson and Herrington 1927). Andrew recommends that iodised salt should be kept in lacquered tins.

Iodised mineral feeding mixtures sold for stock-feeding also lose iodine through catalytic oxidation by iron, copper and manganese, and the addition of a reducing agent together with a soluble pyrophosphate has been found to stabilise the mixture. Another process is to coat the grains of potassium iodide with finely powdered calcium or magnesium stearate, when a free-flowing stable powder is obtained (Johnson and Frederick 1940).

Iodides have been added to public water supplies in several places in the United States and also in England, but this practice has the disadvantage that those who may be particularly susceptible to iodine dosage cannot avoid it.

IODISED WRAPPINGS FOR FRUIT. In 1934 it was found that if imported grapes were wrapped in paper impregnated with a solution of iodine in potassium iodide, wastage due to mould growth in transit could be greatly reduced (Tomkins 1934). It was suggested that not only grapes but tomatoes and possibly other fruit might be similarly treated. Iodine is presumably a preservative within the meaning of the Preservative Regulations 1925–7, and the use of iodised wrappers would therefore be permissible only if the food does not thereby take up an appreciable amount of iodine. Grapes imported experimentally in these wrappings were found to contain up to 40 p.p.m.

Apart from any contravention of existing regulations, no one would suggest that the occasional consumption of half a pound of grapes thus treated, containing, say, 8 mg. (8,000 μg.) of iodine, would be followed by any ill effects. Crystallised and bottled cherries coloured pink with erythrosin (iodofluorescein), one of the United States permitted dyes, also contain about 30 p.p.m. of iodine, although in a somewhat different form. If, however, the use of iodised wrappers were to be extended to a number of different fruits and possibly other foods (and it would be difficult in practice to prevent such extension), the daily intake of iodine from this source might be many times greater than that from iodised salt, and the question of possible ill effects would certainly arise. This method of reducing spoilage of fruit in transit should not be used unless it is clear that the objections advanced by Mühe and others (p. 324) to the habitual ingestion of small quantities of iodine by adults are entirely without foundation. This use of iodine as a fungicide is on a somewhat different footing from that of iodised salt since, as Orr and Leitch point out, if iodised salt is found to have toxic effects it is very easily avoided ; iodine introduced into fruit, etc., unless its presence is disclosed, cannot be avoided. It may be noted that with certain fruits it has been found that iodised wrappers are harmful to the fruit tissue and render it less resistant to attack by moulds (Wardlaw 1936).

Gigli (1936) refers to the addition of tincture of iodine to peas, which makes them retain their original appearance on cooking. This use of iodine has not so far been observed in this country.

THE DETERMINATION OF IODINE IN FOOD

The problem of determining accurately a few micrograms, or fractions of a microgram, of iodine in foods and biological material is one of great difficulty, and many different methods and modifications of them have been devised. They all involve in the first place destruction of organic matter without loss of iodine, separation of iodine from the residue and its concentration into a relatively small bulk. It is then either determined colorimetrically after solution in chloroform or carbon disulphide (von Fellenberg 1923, Hurtley 1929), or it is oxidised to iodic acid which is allowed to react with added potassium iodide, and the iodine set free titrated with thiosulphate (Kendall 1920, Leitch and Henderson 1926, Harvey 1935). The latter method has the advantage that for each atom of iodine in the material under examination six atoms are actually titrated, according to the equation

$$HIO_3 + 5HI = 6I + 3H_2O.$$

It has, however, the disadvantage that, since it is necessary to add potassium iodide, the merest traces of oxidising agents able to liberate iodine from hydriodic acid may completely falsify the result.

The development of these methods has been reviewed by McClendon (1939) and by Harvey (1935). Harvey's method is in general use in this country and is given in outline below. His report should be consulted for full details.

DESTRUCTION OF ORGANIC MATTER. Potassium and sodium iodides are appreciably volatile at temperatures above 500°. Hurtley found, when working with 10 ml. of blood, that if enough potassium hydroxide or carbonate is added (1·5 ml. of 10N KOH or 2 ml. of 80 per cent K_2CO_3 solution) ashing can be carried out in a muffle at 500° without appreciable loss of iodine. Von Fellenberg (1923) and McClendon (1929) prefer not to let the temperature rise above 400° to 450°, but Harvey obtained good results with Hurtley's method, using about 0·8 ml. of 10N potassium hydroxide for each gramme of dry solids in the sample. The carbonised residue is extracted with water and again ignited and extracted until free from carbon. The dishes in which the ashing is carried out must be of pure nickel containing no copper ; the presence of copper may lead to serious losses of iodine.

It is not advisable to add nitrate to facilitate combustion, owing to the danger of local overheating and consequent loss of iodine, and to the formation of nitrite which must be removed before the solution is acidified in case it should liberate free iodine. The possibility that iodide might be oxidised to iodate which would escape the subsequent alcohol extraction has been much debated, but Reith and van Dijk (1937) have shown that no iodate is produced when nitrates are added.

However carefully the open ashing process is carried out it is sometimes impossible to avoid loss of iodine. McClendon has developed a method of oxidation in a closed tube in which the products of combustion are collected in suitable absorbents. By means of a continuous screw feed device large samples of material can be dealt with. This is the logical method of avoiding loss of iodine, but in practice difficulties arise owing to the formation of nitrite, and it is not easy to secure complete combustion. The apparatus used is described and illustrated in McClendon's book (1939), also in the *Biochemical Journal*, 1934, **28**, 1209, the *Analyst*, 1934, **59**, 839, and the *A.O.A.C. Official Methods of Analysis*, 1945, p. 131.

Some analysts have adopted wet combustion methods in which the material is heated with sulphuric and chromic acids containing cerium sulphate as catalyst. This converts iodine into iodic acid. When all organic matter has been destroyed a reducing agent such as oxalic or hypophosphorous acid is added to reduce iodic acid to iodine, which is distilled over and absorbed in alkali (Leipert 1933, Trevorrow and Fashena 1935, Baumann and Metzger 1937). Riggs and Man (1940) oxidise with sulphuric acid and permanganate, with ceric sulphate and copper as catalysts. None of these methods seem to have come into general use.

EXTRACTION OF IODINE FROM THE ASH. In Harvey's method the combined water extract of the ash is evaporated to dryness on a steam bath and ignited at a low temperature. Potassium hydroxide will have been mostly converted to carbonate during the ashing process. The dry residue is dissolved in the minimum amount of water and the solution extracted with absolute alcohol. This dissolves out potassium iodide and at the same time removes some of the water, leaving the residue of potassium carbonate in a pasty condition. The alcoholic solution is evaporated to dryness with precautions to prevent the access of dust, and of air which may by oxidation produce non-volatile aldehyde resins. The slightest trace of organic matter in the residue after evaporation of the alcohol will cause low results, owing probably to premature reduction of iodate at a later stage and consequent loss of iodine. To ensure complete absence of organic matter the residue is heated for two minutes with a very small quantity of potassium nitrate. Any nitrite formed must be removed, since it would interfere with the quantitative oxidation of hydriodic acid to iodic acid by bromine and lead to low results. It is removed by dissolving the residue in a small quantity of sodium azide solution.

McCullagh (1934) recommends distillation at this stage to free the iodine from substances which may interfere with the subsequent titration. He dissolves the residue from the alcohol extraction in water and distils with sulphuric acid, ferric sulphate and hydrogen peroxide, the iodine being absorbed in sulphurous acid or in alkali. This seems, however, to be an unnecessary complication of the process.

OXIDATION OF IODIDE TO IODATE. The solution of the alcohol residue, after treatment with sodium azide, is made just acid with sulphuric acid and boiled with bromine water, which oxidises hydriodic acid to iodic acid. Bromine has been found to be the most satisfactory oxidising agent for the purpose. Freshly prepared chlorine water has been used by several analysts, but has certain disadvantages including the risk of formation of oxy-acids of chlorine which interfere with the final titration. In some of the older methods permanganate was used as the oxidising agent, any excess being destroyed with alcohol, but although applicable to larger quantities of iodine (p. 332) it is unsuitable for the minute traces present in foods. Bromine can be completely removed from the liquid by boiling, without leaving any residues which may interfere with the final titration. The bromine used must be free from traces of iodine.

TITRATION OF IODINE. The solution after bromine oxidation is boiled down to a volume of about 2 ml., cooled, treated with a small quantity of starch solution and potassium iodide and titrated with N/500 thiosulphate solution. A microburette for this purpose may be made from capillary glass tubing of about 0·5 mm. internal diameter and graduated in thousandths of a millilitre. A volume of 0·01 ml. occupies a length of about 5 cm. The capillary tube is widened at one end and joined to a short piece of ordinary glass tubing into which a metal screw is sealed. The whole burette is filled with mercury. The open (delivery) end of the burette is bent over at an angle so that during titration the point can be kept below the surface of the liquid which is being titrated. By dipping the open end into standard thiosulphate solution and turning the screw the capillary tube can be filled with the solution, and by reversing the motion of the screw the smallest quantity can be delivered as required. In burettes of this type, when set up vertically, it is sometimes difficult to make a mercury-tight joint at the junction with the metal screw, but if the lower end of the burette carrying the screw attachment is bent upwards for four or five inches so as to reduce the mercury pressure on the screw, it will be found much easier to get a satisfactory joint. A simple form of microburette is described by Holt and Callow (1942), and Wyatt (1944) gives details of several different types.

In titrating these small amounts of iodine there is a considerable dilution error, and the result will vary with the volume of liquid titrated. The error can be minimised by carrying out the blank test under exactly similar conditions, but in the actual determination the final volume of the titrated liquid is greater than the final volume of the blank. Harvey determines the volumes after titration and applies a small correction to the result. Aitken (1930) concentrates the solution before titration to 0·2 ml. in a specially designed tube and thus avoids the dilution error.

With this method the most convenient amounts of iodine for titration are roughly from 0·5 to 5 μg., which implies that the weight of sample to be taken is about 40 to 50 ml. of milk, 3 to 5 g. of dried vegetables, or 10 ml. of blood. One point particularly stressed by Harvey is the necessity of ensuring that the atmosphere of the laboratory in which the analysis is carried out does not become contaminated with traces of iodine. Failure to obtain low and constant blanks shows that some such contamination is taking place.

With samples containing several parts of iodine per million, as for instance cherries coloured with erythrosin or fruit wrapped in iodised paper, the titration with N/500 thiosulphate can be carried out with an ordinary burette and the extreme refinements of Harvey's method are not so essential.

COLORIMETRIC DETERMINATION OF IODINE. An alternative to titration is to liberate iodine from the residue of potassium iodide by means of nitrite and to transfer the iodine to chloroform or carbon disulphide for colorimetric determination (von Fellenberg 1923). Carbon disulphide is preferable since the partition coefficient for iodine between it and water is 620 compared with 130 for chloroform (Allott, Dauphinee and Hurtley 1932).

For the colorimetric determination the alcohol extract is evaporated to dryness as for the titrimetric method, the greatest care being taken to avoid contamination with organic matter. The residue is taken up with 1 ml. of water, the solution transferred to a small stoppered tube and a small drop of nitrosulphonic acid added. 0·2 ml. of pure carbon disulphide is now introduced into the tube, and after all the iodine has passed into the solvent the colour is compared with that of standards. The method has the advantage that no potassium iodide is added so that there is no risk of obtaining fictitious results from the accidental presence of oxidising substances in the final stage. It is not, however, quite so sensitive as the titrimetric method.

DETERMINATION OF IODINE IN IODISED SALT. For the examination of iodised salt containing 1 p.p.m. or more of iodine simpler methods may be adopted. In the one given by Andrew and Mandeno (1935) bromine water is used for oxidation of iodide to iodate followed by addition of potassium iodide and titration as above. They find that it is very important to control the acidity of the solution. With increasing acidity the conversion of hydriodic to iodic acid in presence of sodium chloride is progressively less complete, and at the same time increasing amounts of iodine are lost, possibly as monochloride. The solution (40 g. of salt in 200 ml. of water) must be made only just acid to methyl orange before being oxidised with bromine, and 2 ml. of normal hydrochloric acid added before titrating with N/500 thiosulphate solution (cf. also Osborn and others 1943). The Association of Official Agricultural Chemists (*J.A.O.A.C.*, 1944, **27**, 110) recommend that the bromine oxidation and thiosulphate titration should be carried out in phosphoric acid solution.

In my experience the following modification of the methods of Bernier and Peron (1911) and of Baughman and Skinner (1919) gives good results with iodised salt.

40 g. of the salt are dissolved in 150 ml. of hot water. Some samples, owing to the presence of magnesium carbonate, do not dissolve to a clear solution. 3 ml. of normal sodium carbonate solution are added, or more if required to make the solution distinctly alkaline. 10 ml. of N/10 potassium permanganate solution are then added, which should make the solution strongly pink. The liquid is boiled for 10 minutes and allowed to cool to between 60° and 70°, and 2 ml. of alcohol are added. It is then again brought to the boiling-point. All permanganate should now have been destroyed and only a brown precipitate remain in the liquid. The contents of the beaker are filtered while hot and the precipitate washed on the filter with hot water. The filtrate and washings are acidified with 1 ml. of glacial acetic acid and boiled again for about 10 minutes. The solution is cooled to room temperature and about 1 g. of pure potassium iodide added. After allowing it to stand for about half an hour, starch solution is added and the free iodine titrated with N/100 thiosulphate solution. Each ml. of N/100 thiosulphate corresponds to 0·00021 g. of iodine in the sample.

Traces of iron or manganese in a sample of iodised salt may lead to high results owing to liberation of iodine from potassium iodide in the titration. Gronover and Wohnlich (1931) find that this may be prevented by the addition of one or two drops of 10 per cent oxalic acid solution. Phosphoric acid has a similar effect (Werner 1930).

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13

[BROMINE]

BROMINE IN PLANT AND ANIMAL TISSUES. In the mineral world the relative proportions of chlorine, bromine and iodine, according to Vogt, are roughly 250 : 1 : 0·1. Similarly in plant and animal tissues bromine occupies a position between chlorine and iodine, but the bromine and iodine concentrations relatively to that of chlorine are smaller, the proportions being, very roughly, 1,000 : 1 : 0·01.

So far as is known bromine has no specific function in plant or animal metabolism, and its normal presence in tissues does not imply that it is in any way essential. In plants the bromine content, like that of iodine, varies greatly with species and locality. Thus cereals may contain from 1 to 8 p.p.m. and most vegetables from a trace up to 5 p.p.m. Fruits in general contain less bromine than vegetables, usually of the order of 1 p.p.m., or less, in the fresh fruit. Many fruits contain none at all. In animal tissues the quantities range from about 1 to 8 or 9 p.p.m. (Neufeld 1936, Ucko 1936), being highest in glands such as the pituitary, thyroid and adrenal. For cows' milk Ford and others (1940) give 1 to 2·5 p.p.m.

The mean bromine content of the human body is calculated to be 1·7 p.p.m. (Damiens 1938). A good deal of this is derived from salt ; Ford found 54 and 120 p.p.m. in two samples of bakery salt.

Several investigators have claimed that the pituitary and other glands contain organic compounds of bromine possessing functional importance, but so far there is no clear evidence of the existence of any hormones of this character (Dixon 1935, Leipert 1938). It has been suggested that the bromine content of blood bears some relation to certain forms of mental disease but this has not been confirmed. Dixon (1935) found the blood bromine to vary between 4 and 13 p.p.m. and Karp and Wolfsohn (1939) 5 to 10 p.p.m. Ucko (1936) found lower values, 1·5 to 3·5 p.p.m., and Conway and Flood (1936) 2·3 to 5·7 p.p.m. Ucko pointed out that after ingestion of drugs which contain bromine it is eliminated very slowly from the body, and that many months may elapse before the blood and tissues show normal figures again. This probably accounts for the high and variable values recorded by some observers. The bromide content of normal blood may be expected to vary with the intake in food. According to Doering (1938) some of the blood bromine is in organic combination.

Marine animals, especially corals, contain in general a higher proportion of bromine than land animals. Mörner (1913) isolated a bromine-containing protein (gorgonin) from the coral *Primnoa lepadifera* and identified the compound 3:5-dibromo-*dl*-tyrosine among the products of hydrolysis of this protein with barium hydroxide. Friedländer (1922) found that the purple dye obtained from the mollusc *Purpura aperta* and other species appeared to be identical with 6:6′-dibromindigotin. Marine algae also contain much bromine, but in many sea plants and animals a high bromine content is due to the fact that they are permeated with sea-water, which contains from 50 to 60 p.p.m. of bromine or about 3·5 parts for every 1,000 of chlorine.

POTASSIUM BROMATE AS A FLOUR IMPROVER. Potassium bromate is widely used as a flour improver. It is added usually at the rate of $\frac{1}{30}$ oz. per 280 lb. of flour, equivalent to 3·6 p.p.m. of bromine in the flour and about 2·5 p.p.m. in the bread (Ford and others 1940). Its action in strengthening and improving the dough is thought to be due to the oxidation of glutathione, which acts as an activator of proteolysis in the dough and tends to weaken it. The precise function of potassium bromate and other oxidising agents is, however, still rather obscure (cf. Jörgensen 1935, Read and Haas 1937, Ziegler 1940). The addition of bromate is not easily detected, since the natural bromine of wheat flour may be from 1 to 8 p.p.m., and an additional 3·6 p.p.m. due to added bromate is usually within the limits of normal variation (Ford 1940). Smith and Geddes (1942), however, refer to the addition of 40 p.p.m. of potassium bromate in some instances, equivalent to nearly 20 p.p.m. of bromine in the flour.

METHYL BROMIDE FUMIGATION OF FOOD. Methyl bromide is widely used in the United States for fumigating foods, especially vegetables and dried fruits. During 1938 and 1939 several thousand car-loads of fresh vegetables were thus treated to destroy Japanese beetle (Dudley and others 1940). Methyl bromide has been found effective against many insects at nearly all stages of development. Its boiling-point is 4·6° so that at ordinary temperatures it is a gas. The limits of inflammability lie within a very narrow range, 13·5 to 14·5 per cent by volume. It is toxic to human beings, causing epileptiform convulsions, nervous symptoms and progressive paralysis with pronounced delayed action. It is particularly dangerous on account of the fact that in low, but still toxic, concentrations it has practically no smell. 1 g. of methyl bromide vapour in one cubic metre of air (1 part in 4,230 by volume) is considered to be a dangerous concentration.

For fumigation of fresh fruit and vegetables it is used at the rate of 2 to 2½ lb. per 1,000 cubic feet of space for 2 hours, for dried fruits 2 lb. per 1,000 cubic feet for 15 to 24 hours and for grain 1 lb. per 1,000 cubic feet for 24 hours (Dudley and others 1940). Various fresh vegetables, fresh and dried fruits, cereals and nuts fumigated in this way and subsequently exposed to air for 24 hours were found to contain the following amounts of methyl bromide in parts per million. The figures are calculated from the difference between the bromine content of the food before and after fumigation, and no allowance is made for hydrolysis of methyl bromide absorbed by the food.

	Methyl bromide parts per million		*Methyl bromide parts per million*
Wheat	Trace	Oats	36
Prunes	Trace	Beetroot	36
Raisins	3	Green beans	44
Apples	4	Flour (white)	55
Potatoes	6	,, (wholemeal)	76
Carrots	15	Cheese	87
Rice	17	Rolled oats	176
Turnips	25	Nut kernels	158–182

Young and others (1943) found varying amounts of residual bromine, up to as much as 247 p.p.m., in flour and cereal products fumigated commercially with methyl bromide. Lubatti and Harrison (1944) have determined the residual methyl bromide in wheat after experimental fumigation, using a method by which methyl bromide in air is decomposed by catalytic oxidation on a heated platinum wire and the hydrogen bromide formed titrated with silver nitrate. They found that after 25 days' storage roughly half of the methyl bromide absorbed was still present unchanged in the wheat and was recoverable by aeration, the other half having reacted with water or other constituents of the wheat.

Absorption of methyl bromide is greater with the more finely divided foods and also with foods containing much fat. The latter may retain considerable amounts even after prolonged exposure to air. It is sometimes stated that methyl bromide residues dissolved in fat or oil are rapidly hydrolysed to inorganic bromide but this would seem very doubtful.

In experiments on animals Dudley found that for rabbits the minimum lethal dose of methyl bromide administered in oil was 60 to 65 mg. per kilogram of body weight. Much smaller quantities in food had an adverse effect upon the rate of growth. Rabbits fed for a year upon grain and hay containing 80 to 90 p.p.m. of methyl bromide showed an increase of weight 10 per cent less than that of controls. Dudley concludes that it seems unlikely that the small amount of methyl bromide or bromine residues in commercially fumigated fresh vegetables and fruits or dried fruits would be harmful to the consumer. This may be so, but it is not always possible to control commercial practice in these matters and over-dosage, or even normal dosage, with methyl bromide of foods containing much oil or fat would be by no means free from risk. The effect of methyl bromide upon growth is thought to be due to inhibition of thyroid function.

BROMINE COMPOUNDS AS FOOD PRESERVATIVES. Monobromacetic acid and allied bromine compounds, e.g. glycol bromhydrin, appear to have been used in France and Holland as food preservatives, especially for wine and fruit juices (Chelle and Vitte 1936, Florentin and Munch 1936, Reith 1940, von Fellenberg 1944). Some of these compounds are stated to be about as toxic as salicylic acid and to have an irritating action on the alimentary mucous membrane. Bromacetic acid resembles iodoacetic acid in its inhibiting action on the sulphydryl group in certain enzyme systems (Dickens 1930). Preservatives of this type are prohibited in this country under the Preservatives Regulations 1925–7.

THE DETERMINATION OF BROMINE IN FOOD

Bromine in food and biological material is almost always accompanied by a large excess of chlorine from which it must be separated. Iodine is usually present in such minute quantities that for practical purposes it can be neglected. The methods in use depend upon the fact that bromides can be oxidised to free bromine or to bromic acid more readily than chlorides to chlorine or chloric acid. Hahn (1935), from a study of oxidation-reduction potentials, maintains that it is theoretically impossible to liberate the whole of the bromine present without at the same time liberating a small quantity of chlorine, and that results which are apparently quantitative are in fact due to a compensation of errors. For practical purposes, however, the oxidation can be made sufficiently selective over a fairly wide range of chloride and bromide concentrations.

DESTRUCTION OF ORGANIC MATTER. Most of the workers in this field have used dry ashing in presence of excess of potassium hydroxide (Francis and Harvey 1933), followed in some instances by alcohol extraction (Dixon 1934). Where bromine is present wholly in inorganic form proteins may first be removed by precipitation with tungstic acid (Yates 1933) or with methyl alcohol (Conway and Flood 1936). In general, the ashing of the material is carried out as for an iodine determination, great care being taken to destroy every trace of organic matter.

OXIDATION OF BROMIDE TO BROMINE

With Chromic Acid. Evans (1931) oxidised bromide in presence of chloride with chromic acid in sulphuric acid. In a series of experiments with sulphuric acid of different strengths from 0·86N to 9·52N he found that the reaction was complete only in the higher strengths of acid (8 or 9N). In practice this strength is most readily attained by taking up the ash with water and adding to the solution one-third of its volume of concentrated sulphuric acid. After adding solid chromic acid a steady stream of air is drawn through the liquid, and the bromine which is carried over absorbed in a receiver containing a measured quantity of standard arsenious oxide solution and sodium bicarbonate. The excess of arsenious oxide is titrated with standard iodine solution. In presence of relatively large amounts of chloride slightly high figures are obtained owing to the liberation of small quantities of chlorine, and to obtain accurate results it is necessary to absorb the bromine in dilute sodium hydroxide solution and subject it to a second oxidation and distillation with chromic acid.

Francis and Harvey (1933), in adapting this method to the determination of minute amounts of bromine in a few millilitres of blood, oxidise the alkaline ash of the sample with a saturated solution of chromic acid in syrupy phosphoric acid (sp. gr. 1·7). The phosphoric acid–chromic acid is first heated and aerated to destroy every trace of organic matter and then slightly diluted with water (about 5 ml. of water to 90 ml. of phosphoric acid), and the final mixture tested on standard solutions of bromide with excess of sodium chloride. Every batch of stock solution must be so tested to ascertain whether it gives a satisfactory result with known amounts of potassium bromide. The reaction is carried out in a train of glass bubblers fitted with ground glass connections, and the greatest care must be taken at all stages to exclude traces of organic matter. The bromine set free is drawn over into dilute potassium iodide solution and the liberated iodine titrated with N/500 thiosulphate. Francis and Harvey call attention in their paper to a number of precautions which must be taken in order to obtain satisfactory results with quantities of bromine of about 5 μg. and upwards. In this method also, it would seem that the results may be to some extent affected by a compensation of errors, since in absence of chlorides they tend to be too low and in presence of excess of chloride slightly high.

Yates (1933) oxidises with chromic acid in approximately 10N sulphuric acid and collects the bromine in potassium iodide solution, the liberated iodine being titrated with N/1,000 thiosulphate. His method is therefore in some degree a combination of those of Evans and of Francis and Harvey. He claims that it is accurate to within 2 μg. for amounts of bromine from 5 to 1,000 μg. A similar procedure is used by Dudley (1939) for determining bromine in foods after fumigation with methyl bromide. The methyl bromide is first hydrolysed with 1 per cent alcoholic potassium hydroxide for 12 hours at room temperature, the alcohol distilled off, and the residue ashed.

Neufeld (1936) found that Francis and Harvey's method gave low results and that Yates' was more satisfactory. He recommended that biological material should be ashed by Francis and Harvey's method and the alkaline ash oxidised as in Yates' method. This procedure gave him very satisfactory results with quantities of bromine between 3 and 900 μg. Conway and Flood (1936) describe a micro-method for determining bromide in blood in which the oxidation is carried out in approximately 7·5N sulphuric acid containing chromic acid.

Oxidation with Permanganate. Permanganate, as is well known, tends to oxidise hydrochloric acid to chlorine, but it is possible by choice of suitable conditions to reduce the oxidation of hydrochloric acid to a negligible amount. Behr, Palmer and Clarke (1930) oxidise bromide to bromine with potassium permanganate in phosphoric acid. In presence of chloride a small quantity of chlorine is produced, but by extracting the solution with carbon tetrachloride, reducing the bromine and chlorine with sodium sulphite and repeating the permanganate oxidation and carbon tetrachloride extraction, the whole of the bromine can be obtained free from chlorine. Hartner (1933) finds that in weak acid solution in presence of manganous sulphate and concentrated sodium sulphate the oxidation of hydrochloric acid is repressed and bromine can readily be determined, after aspiration in a current of air, without interference from chlorine.

Colorimetric Determination. Bromine when set free by oxidation may be determined colorimetrically by the formation of tetrabromfluorescein (eosin) from fluorescein. Hahn (1935) liberates bromine by means of chloramine-T in an acetic acid-sodium acetate buffer solution of pH 5·5 to 5·6 in presence of fluorescein. The reaction is stopped after 1 to 1½ minutes by adding a little sodium hydroxide solution containing thiosulphate to reduce the excess of chloramine-T, and the colour of the solution compared with standards. Iodine if present in appreciable amount will also give a pink colour due to the formation of iodofluorescein (erythrosin). Hardwick (1942) uses this method for the determination of traces of bromine in cerebrospinal fluid. Stenger and Kolthoff (1935) oxidise with calcium hypochlorite in a borax buffer solution at pH 8·7 to 8·8 and determine bromine by the conversion of phenol red into bromphenol blue. Balatre (1936) also uses this method with chloramine-T oxidation.

A third colorimetric method introduced by Denigès and Chelle (1913) depends upon the action of free bromine upon a colourless solution of fuchsine in sulphuric acid. The red bromfuchsine can be extracted with chloroform and the colour compared with standards (Frezouls 1934, Indovina 1935).

In oxidations with chloramine-T or hypochlorite, very careful control of the conditions is necessary to avoid further oxidation of bromine to bromate. These colorimetric methods depend for their accuracy upon securing complete reaction of bromine with fluorescein, phenol red or fuchsine before any of it undergoes further oxidation, and upon preventing the hypochlorite from bleaching the dye.

OXIDATION TO BROMATE. Dixon (1934) determines bromine in 10 ml. of blood by oxidising it to bromic acid with potassium hypochlorite in presence of boric acid and half-saturated salt solution. Excess of hypochlorous acid is removed by adding hydrogen peroxide which in its turn is removed by boiling. On addition of potassium iodide six atoms of iodine are liberated for each atom of bromine and the iodine is titrated with thiosulphate. This procedure, including alkaline ashing of the blood sample, follows the same lines as the determination of iodine. Doering (1937) employs a similar method in which the solution containing bromine is oxidised with calcium hypochlorite, the excess of hypochlorite being removed with sodium formate (cf. also Kolthoff and Yutzy 1937, Brodie and Friedmann 1938). Ford and others (1940) use a modification of this method for determining bromine in flour treated with bromate and find that, when sodium formate is used to remove excess of hypochlorous acid, it is necessary afterwards to add a little ammonium molybdate as a catalyst for the reaction between bromate and potassium iodide, since sodium formate retards this reaction. They give a number of results obtained in two different laboratories using both the Francis-Harvey and the bromate methods, which show satisfactory agreement.

Towner and Koehn (1946) and Hoffer and Alcock (1946) give methods for the detection and determination of bromate in flour, depending on the liberation of iodine from potassium iodide, but the reaction is not specific and is given also by iodate and persulphate.

Stenger and others (1939) use the bromate method for determining methyl bromide residues in fumigated food. 5 to 10 g. of the sample are treated with 3 ml. of saturated aqueous sodium chloride, and 40 ml. of 2 per cent alcoholic potassium hydroxide to hydrolyse methyl bromide. After evaporation of the alcohol the residue is ashed and the ash extracted with 2 per cent aqueous hydrochloric acid. The extract is neutralised and evaporated and the bromine determined by Kolthoff and Yutzy's method (oxidation to bromate with hypochlorous acid). It is claimed that 1 mg. of methyl bromide in 10 g. of flour, nuts or dried fruit can be accurately determined in this way. In a later paper by the same authors (1942) this method is further elaborated and extended to the separate determination of total bromine and inorganic bromine, the difference between these being taken as residual methyl bromide. For the determination of inorganic bromine unchanged methyl bromide is first removed from the sample by extraction with dichlormethane. Lubatti's method for unchanged methyl bromide in food has been referred to above (p. 339).

A simple and ingenious method of determining chloride, bromide and iodide together when they occur in relatively large amounts, e.g. in some mineral waters, is that of Lockemann and Kunzmann (1933). It involves three operations : (*a*) iodine is liberated with ferric chloride and is determined colorimetrically, (*b*) total halogen is determined by titration with standard silver nitrate, (*c*) a known volume of standard silver nitrate, insufficient to precipitate all the halogens, is added : this precipitates silver iodide, silver bromide and some silver chloride. From the weight of this precipitate and the figure obtained in (*a*) above, the amount of bromine is calculated. The amount of chlorine is calculated from (*a*), (*b*) and (*c*). A somewhat similar method is given by Behrman and others (1941).

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14

[FLUORINE]

THE occurrence of fluorine in food and water has recently attracted much attention, since it has been recognised that fluorides and fluosilicates, even in extremely small amounts, may cause profound changes in the structure of bones and teeth.

FLUORINE AND CALCIUM PHOSPHATE. When calcium phosphate comes into contact with water containing fluorides, phosphoric acid is partially replaced by fluorine with the formation of fluorapatite, $3Ca_3(PO_4)_2,CaF_2$. Natural rock phosphates, of which there are large deposits in various parts of the world, contain varying amounts of fluorine up to a maximum of about 3·8 per cent, representing complete conversion to fluorapatite. Apatites of the general formula $3Ca_3(PO_4)_2,CaX$, where X may be $(OH)_2$, Cl_2 or F_2, also enter into the composition of bones and teeth of animals and man. The inorganic part of bone consists essentially of hydroxyapatite, $3Ca_3(PO_4)_2,Ca(OH)_2$, with varying amounts of calcium carbonate (Bassett 1917, Klement and Trömel 1932, Thewlis 1940). Chlorapatite is present in dental enamel to the extent of about 4 per cent, but only 0·2 per cent in human bone, and fluorine in very small amounts. The pathological changes which take place in bones and teeth when fluorides are ingested are associated with an increase in fluorine content, probably in the form of fluorapatite.

FLUORINE IN BONES AND TEETH OF ANIMALS. According to Casares (1930) there is no fluorine at all in the bones of very young animals, but it is present in increasing quantities as the animals grow older, owing to progressive absorption from blood plasma by the hydroxyapatite of bone tissue. Klement (1933) states that the bones and teeth of land mammals contain from 0·03 to 0·06 per cent (300 to 600 p.p.m.) of fluorine, while those of sea animals contain 0·6 to 1·6 per cent. The high fluorine content of sea-fish bones is due to the fact that sea-water contains 1 p.p.m. of fluorine (Goldschmidt).

Roholm (1937) gives the following values for fluorine in bone ash from various domestic animals ; calf 0·015 to 0·038 per cent, ox 0·055, sheep 0·019, pig 0·012 to 0·036 and horse 0·022 to 0·081 per cent. He puts the normal range for the bone ash of terrestrial mammals at 0·01 to 0·1 per cent (100 to 1,000 p.p.m.). Stas (1941) gives 250 to 1,000 p.p.m. for bone ash from Dutch cattle. Figures obtained by me for bone ash, by the method referred to on page 371, are as follows :

	Fluorine p.p.m.		*Fluorine p.p.m.*
Lamb (leg)	28	Pork (leg)	334
,, ,, ...	36	Chicken (leg) . . .	334
Veal (leg)	30	Beef (leg)	1210
,, (loin)	70	,, (rib)	1381
Pork (leg)	283		

According to Boddie (1945) the jaw-bones of normal sheep may contain as much as 0·19 to 0·27 per cent (1,900 to 2,700 p.p.m.).

The addition of mineral salt mixtures containing rock phosphate to cattle foods may greatly increase the average fluorine content of the bones. Another cause of high fluorine content in commercial calcined bone ash is absorption from the products of combustion of coke or other fuel. These points are of some significance in connection with the production from bone ash of acid calcium phosphate for baking powders (p. 358).

Teeth contain, as a rule, less fluorine than bones and the enamel of teeth less than the dentine. Roholm found 0·017 per cent (170 p.p.m.) in the ash of pigs' teeth and 0·008 to 0·034 per cent in that of calves. In one sample of calves' teeth the dentine ash contained 0·022 per cent and the enamel ash 0·0057 per cent (57 p.p.m.). He gives a range of 100 to 400 p.p.m. for the tooth ash of terrestrial mammals.

Similar amounts of fluorine have been found in the teeth and bones of dogs (Rost 1937) and of rats (Bowes and Murray 1935). Boddie (1945) gives considerably higher values for normal sheep's teeth, 0·15 to 0·19 per cent (1,500 to 1,900 p.p.m.).

FLUOROSIS IN ANIMALS. If fluorine is ingested in excess either in food or water, its absorption by the bones becomes very pronounced and may lead to serious enlargement and malformation. Glock, Lowater and Murray (1941) fed rats from the age of six months on food containing 4·7 per cent of fluorine and found that the fluorine of the bones increased from 0·02 to 0·7 per cent, the increase following a logarithmic curve in relation to age. After cessation of fluorine feeding it was gradually eliminated from the bones at a similar rate. This reversal of the storage of calcium fluoride in bones may be analogous to the liberation of fluorine from mineral phosphate under certain conditions, such as contact with salt water (Bromehead 1941 ; cf. p. 354). Fluorine in amounts below 10 p.p.m. in rats' food does not cause symptoms of fluorosis apart from absorption by the bones, but above 10 p.p.m. in the food the teeth begin to show bleaching, and 20 p.p.m. bleaches and elongates the incisor teeth (Evans and Phillips 1939). With larger amounts the incisor teeth show 10 to 25 times the normal fluorine content and become much longer (Hodge and others 1939). The bleaching of the anterior surfaces of rats' incisor teeth is not peculiar to fluorine and may be brought about by other dietary abnormalities, such as lack of certain vitamins, or of magnesium (Moore 1943) or by chronic cadmium poisoning (p. 429).

Chronic fluorosis of cattle and sheep has been known for some time. Maercker, in his book on alcohol manufacture published in 1908, refers to enlargement and malformation of the bones of cattle fed on distillery residues containing fluorides originally added as yeast antiseptics. Bartolucci

(1912) described a similar disease occurring among cattle in the neighbourhood of a superphosphate factory in Italy, due to contamination of the drinking-water by fluorine in the factory effluent. Fluorosis has been noted in the vicinity of aluminium works using cryolite (sodium aluminium fluoride) as raw material and also in the neighbourhood of brick works. The bones of animals affected become thickened and malformed and are chalky white in appearance and more brittle. The fluorine content may range from 0·6 to 1·6 per cent (Blakemore 1944). In Algeria, Morocco and Tunisia, in the neighbourhood of large phosphorite deposits, animals suffer from a disease of the teeth called 'darmous'. The teeth become soft and corroded and eventually wear down so completely that the animals are unable to feed. Similar conditions exist in part of the United States and Northern India. In Iceland periodic volcanic eruptions of fluoriferous ash contaminate grass to such an extent that it causes serious mortality among sheep. Boddie (1945) cites an instance in which pasture containing 40 to 60 p.p.m. of fluorine (0·004 to 0·006 per cent), derived from a neighbouring aluminium factory, caused severe dental fluorosis in sheep.

Symptoms of the more acute types of fluorine poisoning in animals, apart from the bone changes, are retardation of growth, coarseness and roughness of coat, disturbances of the digestive system, nephritis and various forms of paralysis. The use of powdered rock phosphate containing a high proportion of fluorapatite as a mineral addition to the food of dairy cows and other domestic animals is sometimes followed by ill effects of this kind. 0·6 per cent of fluoriferous rock phosphate in the food ration, corresponding to 0·02 per cent (200 p.p.m.) of fluorine, has produced typical symptoms of acute fluorosis. The critical intake for dairy cows and for sheep is given as 2 to 3 mg. of fluorine per kilogram of body weight or about 0·01 per cent in the food ration (Phillips and others 1934, Peirce 1938). For poultry it is higher, 0·035 to 0·07 per cent in the food.

It is noteworthy that the ingestion of aluminium sulphate tends to prevent or lessen the action of fluorine on teeth

(Kempf and Nelson 1937). This may be due to de-ionisation as aluminium fluoride, whereby fluorine is prevented from interfering with calcium metabolism. A high-calcium diet is also stated to afford some degree of protection against fluorosis (Lawrenz and Mitchell 1941).

In cattle suffering from bone fluorosis and moved to another district, elimination of fluorine is rapid for the first three months and afterwards very slow (Blakemore 1944).

FLUORINE CONTENT OF HUMAN BONES AND TEETH. Boissevain and Drea (1933) found 0·13 per cent of fluorine (1,300 p.p.m.) in human bone ash from the eastern United States and 0·7 per cent in that from Colorado Springs, the difference being due probably to the higher fluorine content of water in the latter district. Roholm (1937) found an average of 0·09 per cent for the rib ash of normal adults and about 1 per cent in the bone ash of two workers who had inhaled cryolite dust over a number of years. He gives 0·05 to 0·21 per cent as the normal range for human bone ash, i.e. more than is found in the bone ash of domestic animals (p. 349). Mascheroni and others (1939) found 0·97 per cent in the bone ash of a young woman from a district where the water was high in fluorine. Glock, Lowater and Murray (1941) have examined a number of human rib bones from subjects of different ages and have obtained figures ranging from 0·02 to 0·3 per cent, with no evidence of fluorosis. In general there is a rise of fluorine content with increase of age roughly at the rate of 0·002 per cent (20 p.p.m.) for each year.

In human teeth Hempel and Scheffler (1899) found 0·09 to 0·52 per cent and Trebitsch (1927), using an entirely different method, 0·29 to 0·59 per cent. These figures include both enamel and dentine. Bernardi and Scandola (1937) give the average for dental enamel as 0·38 per cent. Bowes and Murray (1935), however, regard these figures as much too high. They conclude that normal dental enamel in the London area does not contain more than 0·02 per cent of fluorine (200 p.p.m.). Roholm (1937) found 190 to 300 p.p.m. in four samples of the ash from normal teeth. In two instances where dentine and enamel ash were separately examined the dentine showed 300 and 310 p.p.m. and the enamel 44 and 57 p.p.m. He emphasises the fact that dentine contains more fluorine than enamel.

Discrepancies in the figures given by different authorities are due partly to difficulties of analysis and partly to differences in the ages of the subjects, and in their relative exposure to sources of fluorine intake.

MOTTLED TEETH. An affection of the teeth of children, known as ‘ mottled ’ teeth, has been known for many years in various parts of the world, but its cause was obscure until Churchill in 1931 correlated its occurrence with the presence of fluorine in drinking-water (cf. also Smith, Lantz and Smith 1931). Patches of enamel on the teeth appear chalky white and opaque, and eventually a pigment varying from yellow to brown or black is deposited in the defective enamel. The milk teeth are not affected ; the damage takes place in the permanent teeth before they are cut. When the teeth are mature their condition is not influenced by further ingestion of fluorides, but the original damage remains throughout the life of the teeth and may lead to extensive pitting of the enamel. In drinking-water from five areas in which mottled enamel was endemic Churchill found from 2 to 13·7 p.p.m. of fluorine. Ainsworth (1934) and Barr and Thorogood (1934), investigating similar conditions in children's teeth at Maldon in Essex, found 4·5 to 5·5 p.p.m. in the water supply.

Human beings appear to be more sensitive than animals to the effects of fluorine. A number of surveys, carried out for the most part in the United States, indicate that the critical concentration in water for the production of mottling is about 1 p.p.m., corresponding to a daily ingestion of 1·7 to 2·0 mg. Dean (1937) puts the limit lower than this and correlates the incidence of mottled enamel with the amount of fluorine in water, as shown on the following page.

Bromehead and others (1943) have given figures for the fluorine content of over 100 water samples from different counties in England. The greater number of the samples showed from 0·1 to 0·4 p.p.m. and averaged about 0·23 p.p.m. Water from shallow wells in the Oxford clay, Lower Lias clay or Kimmeridge clay, or in the neighbourhood of phosphate deposits in the lower Greensand, is specially liable to contain fluorine. It has been alleged that mild dental fluorosis is far more prevalent in these districts than has hitherto been suspected, but this has not yet been confirmed.

Fluorine p.p.m. in water	*Incidence of mottled teeth*
6	100 per cent, mostly severe.
4	90 per cent, of which about one-third are severe.
1·7 to 1·8	50 per cent, mostly mild.
1	10 per cent, all mild.
0·2	1 per cent, all very mild.

Calcium fluoride is soluble in water to the extent of 16 p.p.m., corresponding to 8 p.p.m. of fluorine, and the solubility increases with rising temperature and carbon dioxide content of the water. Bromehead (1941) suggests that where water containing much salt or other sodium compounds comes into contact with fluorapatite under particular conditions, there may be a reversal of the usual process of fixation of fluorine by phosphates, and fluorine may go into solution as sodium fluoride.

REMOVAL OF FLUORINE FROM WATER. Fluorine can be removed from water by treatment with tribasic calcium phosphate, either as a suspension with subsequent settling or by filtration (MacIntire and Hammond 1938, Nichols 1939, Walker 1940). Adler and others (1938) percolate the water through towers filled with a granular precipitated hydroxyapatite. This material can be regenerated by treatment with 1 per cent solution of sodium hydroxide followed by dilute hydrochloric acid. Ground calcined bone is also effective as a filtering material. One cubic foot of the powder is sufficient for nearly 5,000 gallons of water containing 4 p.p.m. of fluorine. This material also can be regenerated with alkali and acid (Smith and Davey 1939). A mixture of dried aluminium oxide and phosphate is stated to be a very effective precipitating agent for fluorine in water. Greenwood (1940) gives a list of the various substances which have been used for this purpose.

BONE FLUOROSIS IN MAN. The fluorine intake required to produce bone affections in human beings is considerably higher than that associated with mottled teeth in children. The limit has been placed at 4 to 6 p.p.m. in drinking-water, a concentration rarely found in this country. Nevertheless, in a group of English children examined radiologically, interference with the normal course of ossification of the spine was found to be fairly frequent and was commonly associated with dental fluorosis (Kemp, Murray and Wilson 1942). Kemp (1944) points out that there is no evidence that fluorine is solely responsible for these malformations of the spine, but suggests that it may influence the development of such conditions, especially if associated with defective nutrition. Severe malformation of the spine has been reported as occurring in certain districts in India where the water supply contains 3 or 4 p.p.m.

Bone fluorosis is best known as an industrial disease affecting cryolite workers. Cryolite or sodium aluminium fluoride, $3NaF,AlF_3$ or more probably $11NaF,4AlF_3$, is mined at Ivigtut in Greenland and processed chiefly in the United States and at Copenhagen. It is used for the production of aluminium, also in enamel and glass manufacture and occasionally in finely powdered form as an agricultural insecticide. The continued inhalation of cryolite dust by factory workers over a period of several years leads to serious thickening and malformation of the bones, sufficient in the most severe cases to cause almost complete loss of movement in the joints. Various other symptoms occur in greater or less degree (Roholm 1937).

INHIBITION OF ENZYME ACTION BY FLUORINE. Fluorine may inhibit enzyme action, probably as a result of combination with, and immobilisation of, metals such as calcium, copper, iron, manganese and zinc, associated with enzymes. It has been stated that a high fluorine content in blood makes it coagulate more slowly, probably by removal of calcium (Stuber and Lang 1929), but there is some difference of opinion on this point. According to Wulle (1939) normal human blood contains 0·35 to 1·45 p.p.m. of fluorine, or according to Hartmann and others (1940) 0·25 to 0·75 p.p.m. Fluorine in high concentrations may form fluormethaemoglobin in blood, and 10 p.p.m. can be detected by means of its characteristic absorption band at 610 $m\mu$. According to Dufait and Massart (1939) the enzymes inhibited by fluorine include the lipase of ricin, succino-dehydrase, acetylcholine-esterase and esterases in general. The action of fluorine on teeth appears to be governed largely by the calcium and phosphorus content of the blood (Irving 1943), and DeEds (1941) suggests that its effect on calcification may be due to the inhibition of bone phosphatase. Cadmium salts, which also strongly inhibit bone phosphatase, have a similar action on rats' teeth (p. 429). The possible effect of traces of fluorides on the phosphatase of milk, and therefore on the statutory test for commercial pasteurisation, would seem to require further investigation, but it is stated (p. 361) that fluorides in the food of cows do not gain access to the milk, and moreover that milk phosphatase is less sensitive than other phosphatases to traces of fluorides.

Fluorine affects fermentation, probably by forming a complex with the metal of a carrier intermediate between the cytochrome and dehydrogenase systems (Runnström and others 1940). According to Fabre and Brémond (1934) alcoholic fermentation in wine is appreciably inhibited by more than 0·033 g. of barium fluosilicate per litre and still more so by an equivalent quantity of sodium fluosilicate. Fluorides have been largely used as antiseptics in distillery mashes.

FLUORINE AND DENTAL CARIES. A considerable amount of evidence has been accumulated that at certain levels of intake fluorine tends to prevent dental caries. Thus Cox and others (1939), Finn and Hodge (1941) and McClure (1941) have found that when added to the food of young rats it greatly lessens the amount of caries in the teeth. According to McClure a minimum of 10 p.p.m. in rats' food is necessary for partial protection against caries, while 50 to

100 p.p.m. gives complete protection. These larger amounts, however, produce severe fluorosis, as shown by the lengthening of the incisor teeth (Hodge and others 1939).

With human teeth Dean and others (1939, 1942) find that there is less caries in areas where the water supply contains 1 p.p.m. or more of fluorine, in spite of the occurrence of some degree of mottling, than in areas where the fluorine content is low. Among 7,257 children aged 12 to 14 years in 21 American cities there was a clearly defined inverse relationship between fluorine in water and the incidence of caries. Similar correlations have been claimed by Day (1940) for certain districts in India, by Donaldson (1936) for Essex, by King (1940) for the island of Lewis, by Wilson (1941) for Somerset, by Weaver (1944) for North and South Shields, and by Malherbe and Ockerse (1944) for two regions in South Africa.

According to Weaver the optimum concentration of fluorine in water for caries inhibition in man is less than 2 p.p.m. ; no increase in concentration beyond that point will have any greater effect.

The mechanism of this anti-caries action is obscure. Fluorides injected into the blood have apparently no effect in preventing rat caries, but a 6 per cent solution of potassium fluoride applied to the surface of the teeth once a fortnight will produce a 50 per cent reduction in its incidence (Sognnaes 1941). McClure suggests that the action is due to an anti-enzymatic effect of fluorides in the mouth and refers to a statement by Miller (1938) that iodoacetic acid, also an active anti-enzymatic substance, will likewise inhibit caries when added to food. In this connection the observation of Robison and Rosenheim (1934) is interesting, that both fluoride and iodoacetate in extremely low concentrations have a pronounced inhibiting effect on calcification *in vitro*. Iodoacetic acid probably acts by immobilising the sulphydryl group which plays an essential part in many enzyme systems.

The anti-caries effect of fluorides may be due to surface absorption of small amounts by the hydroxyapatite of the enamel with the production of a thin protective layer of acid-resisting fluorapatite. This would be analogous to the formation of fluorapatite geologically, when calcium phosphate is exposed to water containing traces of fluorine. Such absorption might take place from food or water containing minute amounts of fluorides. Thus the mechanism of the anti-caries action of fluorine may be of a kind entirely different from that which produces mottling of children's teeth, although both may occur together at certain levels of fluorine intake (Volker 1939, Cheyne 1940, Wills 1940, Arnold and McClure 1941, Sognnaes 1941, Bibby 1943, 1944). On the other hand we have the observations of Glock and others (p. 350) and Blakemore (p. 352) that fluorine may be partially eliminated from fluorapatite in contact with solutions containing little or no fluorine under certain conditions, in which case one would not expect the formation of a surface layer of fluorapatite unless the teeth were in contact with relatively strong concentrations of fluoride. Possibly there is a tendency towards a state of equilibrium between fluorapatite and soluble fluoride.

According to Armstrong (1935) the average fluorine content of sound teeth is 111 p.p.m. and of carious teeth 69 p.p.m.

FLUORINE IN FOOD

Baking Powder and Self-raising Flour. Acid calcium phosphate of the quality required for baking powder or self-raising flour is normally made either from bone ash or from pure phosphoric acid, but the cheaper grades, especially in times of short supply, are often made direct from rock phosphate containing a high proportion of fluorapatite. Of late years rock phosphate seems to have largely replaced bone ash.

It is possible on a laboratory scale to remove the whole of the fluorine from fluorapatite by calcination at a high temperature with silica in the presence of steam (Elmore and others 1942), and partial defluorination of phosphates for animal feeding is carried out commercially on these lines. Normally, superphosphate for agricultural use as a fertiliser contains one-third to one-half of the fluorine originally present as fluorapatite in the rock phosphate (cf. MacIntire, Hardin and Hammond 1941). For the production of acid calcium phosphate the process usually adopted is to treat the finely ground rock phosphate with dilute sulphuric acid, separate the calcium sulphate formed and concentrate the phosphoric acid solution by evaporation. During these operations it should be possible to eliminate most of the fluorine of the original rock phosphate. The phosphoric acid is then treated with lime or with calcium phosphate, which may either be bone ash or else some of the original fluoriferous rock. If the latter is used, fluorine is of course put back again and the final product may contain anything up to 0·3 or 0·4 per cent (3,000 to 4,000 p.p.m.) or even more. If lime is used the fluorine in the finished product should not be more than is unavoidably retained in the phosphoric acid. Bone ash itself may contain up to 0·1 per cent or more of fluorine (p. 349) which, unless steps are taken to remove it, may represent about 400 p.p.m. in the final product.

In Other Foods. Until recently, few trustworthy figures for the natural fluorine content of plants and of vegetable foods were available. Many analyses were carried out by various workers about thirty or forty years ago, mostly by modifications of the etching test, but in general the results were rough approximations. More recently Phillips and others (1935) have found that the fluorine content of crops from soil treated with fluoriferous mineral phosphates over a long period is not greater than 2 p.p.m. Reid (1936), in an investigation on Chinese foods, found from 1·2 to 8·5 p.p.m. in vegetables, 4·0 to 6·7 p.p.m. in soya bean and 1 p.p.m. in cereals. The amounts found seemed to be independent of locality. Lockwood (1937) found *nil* to 2 p.p.m. in a number of foods derived mainly from vegetable sources. Dahle (1936) gives average figures of 1·2 p.p.m. for apples, 0·65 for lettuce, 0·75 for tomatoes, 1·1 for flour, 1·5 for liver and 0·8 p.p.m. for egg-yolk. Winter and Butler (1933) found considerably larger quantities, 9 p.p.m. in rice, 6 to 8 p.p.m. in spinach and 5 to 10 p.p.m. in cereals, but it is doubtful whether Willard and Winter's original distillation method as used by them is applicable without modification to such small amounts as are found naturally in foods (p. 370). A few miscellaneous food samples examined by a sub-committee of the Society of Public Analysts (1944), during the progress of work upon a standard method of fluorine determination, showed the following figures; beer 0·45, 0·25 p.p.m., canned pilchards 5·8, canned shrimps 2·4, corned beef 0·4, dried egg 0·3, evaporated milk 0·15, milk 0·17, rolled oats 0·6, soya grits 0·6, spinach powder 70·0, split peas 0·4, whisky 0·03 p.p.m. The high figure for spinach powder is remarkable and may possibly be due to the fuel used in drying. McClure (1939) gives the average content for vegetables as 0·8 p.p.m.

Sea fish may contain relatively large amounts, of the order of 5 to 10 p.p.m., derived from the fluorine in sea-water (approximately 1 p.p.m.). Harvey (1945) gives figures ranging from 3·1 to 8·9 p.p.m. for five brands of fish paste.

Clifford (1945) gives the fluorine content of a number of American foods. With the exception of fish and fish products very few of the foods examined contained more than 1 p.p.m. and most of them showed less than 0·3 p.p.m.

Some growths of tea, especially China tea, have a high and very variable fluorine content up to 100 p.p.m. or more in the dry leaf (Cheng and Chou 1940, Lockwood 1937). Reid gives much higher figures, up to 1,750 p.p.m., but it is possible that these were due partly to the presence of manganese in tea (p. 306) and consequent evolution of chlorine on distillation. About two-thirds of the fluorine in tea goes into the infusion. A fluorine content of 80 p.p.m. for tea would imply a daily intake of roughly 1 mg. of soluble fluorine for adults, but much less for children. This source of fluorine must be taken into account in calculating the total intake from food (p. 365).

Gelatin has been reported to contain fluorine owing to the use of sodium fluoride and fluosilicate as additions to salt in the curing of hides. Robertson and others (1934) found up to 200 p.p.m. in ordinary edible gelatin and 160 p.p.m. in gelatin from hides cured with the addition of fluorides, from which they concluded that the use of fluorides for curing did not affect the gelatin for edible purposes. Stuart and others (1937) found only 4 to 10 p.p.m. in gelatin from fluoride-cured hides. Lockwood (1937) found none at all in the gelatin examined by him. Moiseev (1941) gives 1·25 p.p.m. as the average figure for edible gelatin.

Milk contains from 0·05 to 0·25 p.p.m. which is not increased by addition of fluorides to the cows' food (Evans and Phillips 1939, Phillips and others 1934). The casein from milk normally contains about 0·05 p.p.m., but Evans and Phillips found many commercial samples with fluorine contents varying from 4 to as much as 360 p.p.m. Egg-yolk has been stated by several workers to be high in fluorine; Lockwood found only 2 p.p.m. He also found 2 p.p.m. in beef.

Wine may contain from 3 to 28 p.p.m. (Johnson and Fischer 1935). Fabre and Brémond (1934) consider that 5 p.p.m. in wine is normal and that any more is due to the use of fluoride antiseptics or insecticides. It may be noted that this figure is the same as that for Maldon water, which causes mottling of children's teeth (p. 353).

Crossley (1944) gives figures for fluorine in beer derived from the coal used in malt drying. The fluorine in malt ranged from 3 to 15 p.p.m. and in beer from 0·4 to 0·8 p.p.m. He gives reasons for supposing that the stronger beers of peace time may show as much as 2·3 p.p.m., or more in exceptional instances.

Fluorides and fluosilicates were formerly used as food preservatives, especially for the prevention of mould growth in butter, but this practice appears to be almost obsolete and, moreover, it is prohibited in this country under the Preservatives Regulations 1925–7. Schloemer and Zeglin (1938) refer to an outbreak of poisoning from apricot pulp preserved with fluoride, characterised by the onset of acute gastro-enteritis in 15 to 20 minutes. They consider that 50 to 70 mg. of fluorine would be sufficient to cause symptoms. The preservative action of fluorides may be due to the affinity of fluorine for certain metals and consequent immobilisation of metals associated with bacterial respiration and enzyme action (p. 355).

Fluorides have occasionally found their way accidentally into food with serious results. On one occasion pancakes prepared with sodium fluoride in mistake for sodium bicarbonate caused severe illness. The amount of sodium fluoride ingested was about 230 mg., corresponding to 100 mg. of fluorine. A similar occurrence is recorded by Ingraham and Flood (1943). Lidbeck and others (1943) give an account of a severe outbreak of fluorine poisoning in a hospital owing to the use of sodium fluoride cockroach powder in place of dried milk for cooking scrambled eggs.

Fluorides nearly always form an important ingredient of hollow-ware enamel, in which they act as opacifying agents, and may occasionally gain access to food from this source if the enamel is too ‘ soft ’ and is corroded by acids (cf. p. 210). Coste and Garratt (1935) found 30 p.p.m. of sodium fluoride in the acid extract from an enamelled vessel of this kind. Another possible source of fluorine in foods is the refrigerant ‘ Freon ’ (difluoro-dichloromethane), traces of which may be present in the atmosphere of refrigerating chambers (Baker 1935, Yant 1933).

According to Machle and others (1939) the mean daily urinary excretion of fluorine by human beings is about 1 mg. per litre, most of which is derived from food. McClure and Kinser's estimate (1944) for districts with a fluorine-free drinking-water is 0·3 to 0·5 mg. daily.

Insecticides as a Source of Fluorine in Food. Fluorides and fluosilicates are effective insecticides. Markovitch (1928) and Dubroscky (1935) attribute their toxic action to the removal of calcium from the tissues as calcium fluoride. Soluble fluorides cannot be used on plants as they cause damage to foliage, but fluosilicates, especially barium fluosilicate, are used as substitutes for lead arsenate. Sodium fluosilicate is stated by Markovitch to be more toxic than sodium arsenite to insects, possibly on account of their low calcium content, but less toxic to human beings (cf. also Smyth 1932). Powdered cryolite is also used as an agricultural insecticide.

Wichmann and Dahle (1933) found 2 p.p.m. of fluorine in apples which had been subjected to treatment with a fluorine insecticide. Cabbage contained 3 p.p.m. in the inner leaves and 34 p.p.m. in the outer ones, and celery 3·6 to 7·6 p.p.m. with 77 to 135 p.p.m. in the leaves.

ABSORPTION AND RETENTION OF FLUORINE FROM FOOD. The relative toxicity to human beings of soluble and insoluble fluorides seems to depend upon the amount ingested. At high levels of intake calcium fluoride is stated to be far less toxic than sodium fluoride (Markovitch and others 1937, Smith and Leverton 1934), but at low levels, such as are usually met with in food, the form in which fluorine exists in the food, and the relative solubility of its different compounds, probably make little difference to its absorption and toxicity (Lawrenz and others 1939, DeEds and Thomas 1934). With rats there is not a great difference in toxicity of fluorine in different forms of combination when the amount required to produce the first discernible effect upon the teeth is the basis for comparison (Smith and Leverton 1934). DeEds (1942) found that fluorine in dicalcium phosphate was just as active physiologically as that of sodium fluoride.

Machle and Largent (1943) in experiments on one human subject with daily doses of 6 mg. of fluorine in different forms found the following amounts of absorption ; sodium fluoride 97 per cent, calcium fluoride in solution 95·5 per cent, cryolite 77 per cent, solid calcium fluoride 62 per cent, bone meal 37 per cent. The low absorption from bone meal was probably due to the large excess of calcium present. Fluorine normally contained in food was absorbed to the extent of 82·5 per cent.

McClure and others (1945) in balance experiments on five young men receiving 3·0 mg. of additional fluorine daily in the form of sodium and calcium fluorides mixed with food confirmed Machle and Largent's finding that at these low levels of intake about 60 per cent of the fluorine in calcium fluoride, and roughly 40 per cent of that in bone meal, is absorbed from food.

Retention of fluorine normally absorbed from food is very small. In a human subject ingesting daily during six months an average amount of 0·15 mg. in food and 0·3 mg. in drink, the average daily excretion was 0·38 mg. in urine and 0·04 mg. in faeces, indicating a high rate of absorption, but at the same time a very small retention (Machle and others 1942). At higher levels of intake both urinary excretion and retention increase: with a daily intake of 6 mg. of fluorine, in whatever form, roughly half of the amount absorbed is retained (Machle). Lawrenz carried out experiments on rats with cryolite, which is about 23 times more soluble in water than is calcium fluoride. She found that at a level of 10 p.p.m. of fluorine from cryolite mixed with food, retention was only 20 per cent less than from a similar amount of cryolite in water. Her figure for fluorine retention, about 40 per cent, agrees closely with that obtained by Machle for a human subject receiving 6 mg. of cryolite daily.

McClure and others (1945) in their balance experiments referred to above, found that with intakes up to about 4 or 5 mg. of fluorine daily, practically the whole of it is eliminated and there is no retention in the body. Of the fluorine absorbed roughly three-quarters is excreted by way of the kidneys and roughly one-quarter in perspiration through the skin. Thus at low rates of intake, whatever the form in which the fluorine is administered, there is a high rate both of absorption and of excretion, and there is little likelihood of injury to health in adults ingesting not more than 4 to 5 mg. daily. McClure's findings in respect of absorption and retention at this level are somewhat at variance with those of Machle referred to above, but the significance of these experiments lies in the fact that the absorption of fluorine from food at low levels of intake is found to be affected so little by the solubility of the fluoride, a point which has been much debated in connection with acid calcium phosphate. It should be noted that the experiments were carried out on adults and that the susceptibility of young children to fluorosis is probably much greater.

LIMITS FOR FLUORINE IN FOOD. Fluorosis in human beings, if we except that due to industrial occupations, has so far been associated only with fluorine in water supplies. Although the experiments referred to above indicate that fluorine in food, whether soluble or insoluble, is also absorbed, no evidence has been forthcoming that the use of fluoride and fluosilicate preservatives in former years, or of fluoriferous phosphates in baking powders and self-raising flour, or of foods dried in contact with furnace gases, has actually been responsible for human dental fluorosis. It must be borne in mind, however, that these sources of fluorine are intermittent and in the aggregate probably do not raise the total intake to a point where damage to teeth is sufficiently obvious to demand investigation. Laurenz, Mitchell and Ruth (1940) obtained evidence that, for similar amounts of fluorine over a given period, retention is less with intermittent than with continuous doses. The possibility that fluorine may be absorbed from food in amount sufficient to produce dental fluorosis is indicated by Sognnaes' observation (1941) that in Tristan da Cunha nearly 16 per cent of the inhabitants have mottled teeth, although the water contains only 0·2 p.p.m. of fluorine. He suggests that this is due to a fish diet.

As already indicated, the critical concentration of fluorine in water above which there is danger of its causing dental fluorosis in children, appears to be about 1 p.p.m. Assuming an average intake of 3 pints daily the amount of fluorine thus ingested is 1·7 mg., or perhaps about 1·2 mg. for children between the ages of 5 and 7. Water containing less than 1 p.p.m. does not seem to produce appreciable mottling. As far as is known at present the teeth after dentition is complete are not adversely affected by moderate amounts of fluorine, but only in the formative stages. The safety limits of fluorine intake for adults are therefore probably much higher than those for children, but it is obviously children who must receive the first consideration in this matter. Perhaps a total daily intake of 2 to 3 mg., not more than half of which is derived from water, may be assumed to be below the limit of toxicity for young children in so far as dental fluorosis is concerned. More knowledge is required of the amount of fluorine in different foods, and the effect of food constituents such as calcium phosphate upon its absorption. It is not yet clear whether the administration of very small amounts of fluorides, or their external application to the teeth, afford a practicable means of reducing substantially the incidence of caries.

Under the Fluorine in Food Order, 1947, the following limits for fluorine in certain foods have been prescribed.

1. Calcium acid phosphate, sodium acid pyrophosphate or any other acidic phosphate : 300 parts per million.
2. Any article of food (not included in items 3 and 4 below) containing calcium acid phosphate, sodium acid pyrophosphate or any other acidic phosphate and intended for use in the composition or preparation of food : 300 parts per million parts of the calcium acid phosphate, sodium acid pyrophosphate or other acidic phosphate present.
3. Baking powders, including golden raising powder : (*a*) 100 parts per million parts of the article of food where such article yields less than 15 per cent of available carbon dioxide ; (*b*) 133 parts per million parts of the article of food where such article yields not less than 15 per cent of available carbon dioxide.
4. Self-raising flour or any similar mixture (not included in item 3 above) containing a farinaceous substance and an acidic phosphate : 8 parts per million parts of the article of food.

Allowing for a maximum daily consumption of about 100 g. of self-raising flour or an equivalent quantity of baking powder, these limits would imply the addition of 0·8 mg. of fluorine to the daily diet. This quantity, taken in solid food, is not by itself objectionable, but anything much higher might have an appreciable effect, especially in localities where a tendency towards dental fluorosis already exists. The tolerance in the United States for fluorine in fruit and vegetables treated with fluorine insecticides, applicable to interstate commerce, is 0·02 gr. per lb. (2·8 p.p.m.), but for apples and pears this seems to have been increased lately to 0·05 gr. per lb. (7 p.p.m.) (*Federal Register*, **9**, 11836, Sept. 29th, 1944).

THE DETERMINATION OF FLUORINE IN FOOD

The accurate determination of very small amounts of fluorine in food has only recently been achieved. A number of methods are available for larger quantities of the order of 0·05 per cent and upwards, such as may be due to fluoride preservatives or insecticides or to acid phosphates prepared from fluorapatite, but until the publication in 1933 of Willard and Winter's method it was hardly possible to determine quantities of the order of a few parts per million in foods, quantities which may be of significance in connection with fluorosis. Many of the results obtained by the older methods of analysis are far too high.

ZIRCONIUM-ALIZARIN METHOD. For the determination of fluorine in water, de Boer (1925) used the reddish-purple zirconium-alizarin lake, the colour of which is discharged by fluorine with formation of non-ionised zirconium fluoride. In pure solutions of fluorides or fluosilicates the rate of fading is proportional to the amount of fluorine present. This method was used by Barr and Thorogood (1934) for the determination of fluorine in water suspected of causing dental fluorosis. The reagent contained 0·053 per cent of zirconium oxychloride and 0·005 per cent of sodium alizarin monosulphonate. 1 ml. of this solution added to 50 ml. of water gives a faint pink which is discharged by small quantities of fluorides. A rough approximation was first made by comparing the rate of fading of the sample with that of solutions containing, say, 1, 2, 4, 6 and 8 p.p.m. of fluorine, and subsequently an accurate determination by narrowing the range of fluorine content of the comparison solutions. Barr and Thorogood claimed that in this way the fluorine content of a sample of potable water with 5 p.p.m. of fluorine can be determined with an accuracy of 0·1 p.p.m.

A number of ions, $\mathrm{Ca}^{\cdot\cdot}$, $\mathrm{Ba}^{\cdot\cdot}$, $\mathrm{Fe}^{\cdot\cdot\cdot}$, $\mathrm{Al}^{\cdot\cdot\cdot}$, SO_4'', PO_4''', etc., may react either with fluorine or zirconium and interfere with the accuracy of this method. If they are present in appreciable amount care must be taken to use for comparison a solution containing roughly similar amounts of the interfering ions. In most potable waters the mineral salt content is not sufficiently high to interfere seriously with the determination (Ford 1940). Crossley (1944) has still further developed the method and applied it to the determination of fluorine in coal.

Bowes and Murray (1935) have adapted the colorimetric zirconium-alizarin method to the determination of fluorine in teeth. The ashed teeth are fused with sodium carbonate, the melt extracted with water and the solution treated with silver nitrate to precipitate phosphates while leaving silver fluoride in solution (cf. Chancel 1859). The precipitate is well washed and the filtrate treated with sodium chloride to remove excess of silver. In the filtrate from the precipitation the chlorine content is determined and an exactly equivalent quantity of sodium chloride added to the comparison solution. Sulphate is also determined and removed by addition of the exact amount of barium nitrate. In this way the concentration of any ions likely to affect the determination is the same in the test solution and in the comparison solution.

THORIUM TITRATION. As an alternative to measuring the rate or extent of bleaching of a zirconium-alizarin lake, fluorides may be titrated with standard thorium nitrate solution using a very dilute solution of zirconium nitrate and sodium alizarin sulphonate as an indicator (Willard and Winter 1933). To the solution containing fluorides or fluosilicates are added a few drops of the zirconium-alizarin solution, and the pink colour is just discharged by careful addition of dilute hydrochloric acid. On addition of standard thorium nitrate solution the fluorine combines with thorium to form un-ionised thorium fluoride, the end-point of the titration being shown by the re-appearance of the pink colour of the zirconium-alizarin lake. The reaction is,

$$2H_2SiF_6 + 3Th(NO_3)_4 + 4H_2O = 3ThF_4 + 12HNO_3 + 2SiO_2$$

In later work the zirconium nitrate has been omitted as superfluous, since the end-point of the reaction is equally well shown by the formation of the bluish-red thorium-alizarin lake (Shuey 1934).

An alternative method of titrating fluorine solutions is given by Scott and Henne (1935). They use cerous nitrate in place of thorium nitrate and a mixture of methyl red and bromcresol green as indicator, the end-point being shown by the red colour produced with the indicator by free cerium ions in solution. This method does not appear to be widely used. Clifford (1941) has given a method which depends on the bleaching action exerted by fluorine on the reddish-purple aluminium lake of aurin tricarboxylic acid.

DISTILLATION METHODS. In Bowes and Murray's method for fluorine in teeth referred to above, interfering ions are either removed or their concentration in test and control solutions equalised. This troublesome process can be avoided by distillation of fluorine from acid solution either as silicon tetrafluoride or as hydrofluosilicic acid.

When this method is applied to foods the sample must first be ashed at a low temperature with the addition of some substance which will act as a fixative for fluorine and prevent it from being lost on ignition. Calcium hydroxide in the form of milk of lime is probably the best for this purpose (Clifford 1941). Aluminium nitrate may also be used (Dahle 1936), but tends to retard the disengagement of fluorine on distillation. Crutchfield (1942) recommends magnesium acetate. Sodium hydroxide or carbonate, unless in very large excess, does not always prevent loss of fluorine on ignition.

Calcium carbonate and oxide, even of the purest grades obtainable commercially, usually contain fluorine in quantities of the order of 5 p.p.m. or more. Calcium carbonate can be obtained practically free from fluorine by fractional precipitation with ammonium carbonate as follows (*Analyst*, 1944, **69**, 243): 200 g. of the purest granulated calcium chloride available are dissolved in 600 ml. of distilled water. In another 600 ml. of distilled water are dissolved 110 g. of ammonium carbonate and 55 ml. of 0·880 ammonia, and 20 ml. of this solution are added to the calcium chloride solution gradually with stirring. The mixture is brought just to boiling-point, the precipitate allowed to settle and the liquid separated by filtration through a Buchner funnel. The precipitate is rejected. This treatment with 20 ml. of the ammonium carbonate solution is repeated three times, the precipitate in each case being rejected. Finally the clear filtrate is treated with the remainder of the ammonium carbonate solution and the mixture brought just to boiling point and filtered as before. The precipitate of calcium carbonate, now free from fluorine, is washed with hot water until the washings no longer give a reaction for chloride, and dried at 100°. It is ignited to oxide in a platinum dish in quantities of 1 to 2 g. as required.

In the older distillation methods the anhydrous ash of the material was heated with concentrated sulphuric acid and glass fragments, and fluorine volatilised as silicon tetrafluoride SiF_4, with precautions against access of moisture. Working in this way Fresenius in 1866 absorbed the silicon fluoride in water in weighed absorption tubes and measured the increase in weight. Hempel in 1899 measured the volume of the silicon fluoride gas in a burette over mercury. Armstrong (1933) used a specially designed distilling flask and absorbing vessel with ground glass connections. The silicon fluoride was absorbed in water as hydrofluosilicic acid and the fluorine determined colorimetrically. The complicated nature of the distillation and absorption apparatus used in this method is a disadvantage for general work.

In Willard and Winter's method fluorine is distilled, not as silicon tetrafluoride, but as hydrofluosilicic acid from an aqueous solution containing sulphuric, perchloric or phosphoric acids. Perchloric acid is preferred owing to the difficulty of preventing traces of sulphuric or phosphoric acid from being carried over into the distillate and interfering with the titration.

This method, as modified and revised by various workers, is accurate and sensitive for small quantities of fluorine in foods and similar materials. Its latest form is described in a report of a sub-committee of the Analytical Methods Committee of the Society of Public Analysts (1944). The main features of the method are (i) ashing of the sample at a low heat with the addition of pure lime prepared by the precipitation process given on p. 370, (ii) steam distillation with perchloric acid at 138°–140°, glass fragments being added as a source of silica, and silver sulphate to prevent hydrochloric acid from passing over into the distillate, (iii) adjustment of acidity in the distillate and ‘ back-titration ’ of fluorine with thorium nitrate, using alizarin sulphonic acid as indicator. The method is capable of determining quantities of fluorine of the order of 1 μg. to within 0·15 μg. per gramme of material, i.e. 0·15 p.p.m. To obtain accurate results with small amounts of fluorine it is necessary to follow closely the procedure recommended by the committee. For full details the reader is referred to the committee's report. A similar method has been tentatively adopted by the Association of Official Agricultural Chemists (*Official Methods* 1945).

In the steam distillation with perchloric acid it is not advisable to allow the temperature to rise much above 140°, since at 142° concentration of the acid begins and if this is carried too far there may be a risk of explosion (Dahle and Wichmann 1937). If the sides of the flask are overheated free chlorine may be produced which interferes with the titration by bleaching the indicator. This may be avoided by resting the flask on a circular opening in a sheet of asbestos board as recommended in the committee's report. The flask must fit closely in the opening. With foods containing manganese it is possible that traces of free chlorine may be produced, but the addition of silver sulphate should prevent this. Clifford (1941) suggests the addition of a small quantity of hydroxylamine to the distillate to reduce chlorine. The presence of gelatinous silica on the sides of the flask is said to retard the evolution of fluorine, possibly owing to the formation of a non-volatile silicon oxyfluoride (Reynolds and Jacob 1931). The flask should be frequently washed out with hot 10 per cent sodium hydroxide solution to remove any deposit of silica. Large amounts of aluminium in the ash also retard the volatilisation of fluorine, and it may be necessary to distil first with sulphuric acid at about 150°, concentrate the distillate after neutralisation and distil again with perchloric acid (Dahle and Wichmann 1936). With the exception of alum baking powders, no foods are likely to contain enough aluminium to affect the determination.

With materials high in phosphate, such as acid calcium phosphate or baking powder, traces of phosphoric acid may be carried over into the distillate and upset the titration by combining with thorium. The same applies to traces of sulphuric acid. With food materials consisting largely of phosphates or sulphates it may be necessary therefore to evaporate the distillate after neutralisation and redistil with perchloric acid. The procedure is given in an Addendum to the Report of the Committee (1945).

The glass fragments used in the flask must of course be free from fluorine. Opal glass and glass wool often contain it.

ALTERNATIVE COLORIMETRIC METHODS: PEROXIDISED TITANIUM. Several other methods have been used which depend upon the removal by fluorine of metals from their coloured compounds, and consequent discharge of the colour, but in general they are not so sensitive as the thorium-alizarin method. Ferric thiocyanate (Foster 1933) and ferric acetylacetone (Armstrong 1933) are both bleached by traces of fluorine, and the reaction is stated not to be affected by small amounts of neutral salts. Incidentally, this affinity of fluorine for metals should be borne in mind in all work involving their colorimetric determination.

Steiger in 1908 determined fluorine by its action in discharging the orange-brown colour of peroxidised titanium solutions, and this method has been widely used. Wichmann and Dahle (1933) use it for fluorine in water, in phosphates, in spray residues on fruit, etc., in conjunction with a perchloric acid distillation. The bleaching effect is greatest at pH 1·5 and is affected by aluminium, iron, phosphates and sulphates. Suitable concentrations of fluorine for colorimetric comparison are between 0·01 and 0·05 mg. in 50-ml. Nessler cylinders. The reagent is an acid solution of titanium chloride treated with hydrogen peroxide. The procedure has been subsequently modified and improved by Dahle (1937). Details of these methods are given in the *Analyst*. The peroxidised titanium method is useful for the routine examination of butter samples for the presence of fluorides or fluosilicates added as preservatives (Monier-Williams 1912) in preference to the more troublesome etching test.

Fenton (1908) showed that dihydroxy-maleic acid, COOH.C(OH) : C(OH).COOH, gives an intense reddish-brown colour with quadrivalent titanium compounds which is at once destroyed by hydrofluoric acid. The reaction is more delicate than that with hydrogen peroxide and might be of considerable value in the detection and determination of traces of fluorine.

PRECIPITATION METHODS. Fluorine when present in any considerable amount as alkaline fluoride in solution is usually determined by precipitation as calcium fluoride together with calcium carbonate. The precipitate is extracted with acetic acid after ignition and the residual calcium fluoride weighed. Clarke and Bradshaw (1932) find that by paying careful attention to the conditions under which the calcium fluoride is precipitated and also to the method of filtering, good results can be obtained in absence of carbonate. The solution must of course contain no phosphate. If phosphate is present it can be removed as silver phosphate by precipitation with silver nitrate and silver carbonate, excess of silver being removed from the filtrate as chloride (Chancel 1859, cf. also Bowes and Murray, p. 368). Balavoine (1933) precipitates fluorine with barium acetate. It may also be precipitated as gelatinous thorium fluoride (Pisani 1916), which is then ignited to oxide, but thorium fluoride is to some extent soluble in excess of thorium nitrate solution, so that the precipitation must be carried out in such a way that thorium nitrate is never present in more than 25 per cent excess of the amount required for precipitation (Gooch and Kobayashi 1918). Fluorine may also be precipitated with lanthanum acetate in presence of acetic acid and ammonium acetate. The flocculent precipitate of lanthanum fluoride always contains adsorbed lanthanum acetate. On being heated and allowed to stand, it becomes granular and can readily be filtered. It is dried at 130° and weighed. On ignition the acetate is converted into oxide while the fluoride remains unchanged. From the weight of the dry precipitate and the loss of weight on ignition the amount of fluoride present can be calculated (Meyer 1924, Meyer and Schulz 1925). This method is obviously not capable of great precision; Bowes and Murray (1935) found it quite untrustworthy for fluorine in teeth. Small amounts of fluorine can be determined nephelometrically with a 2 per cent solution of lanthanum nitrate in presence of ammonium acetate and acetic acid (Giammarino 1937).

Starck's lead chlorofluoride method (1911) is suitable for larger quantities of fluorine and has been used by Graham (1937) for the analysis of mineral insecticides such as barium fluosilicate. The solution containing fluoride is neutralised and mixed with a large volume (200 ml.) of a nearly saturated solution of lead chloride, and the mixture allowed to stand overnight. The precipitate of PbFCl is filtered off on a Gooch crucible, washed with lead chloride solution and finally three or four times with a small quantity of water. It is dried at 140° to 150° for two hours and weighed. At 18°, 100 ml. of water dissolve 32 mg. and 100 ml. of saturated lead chloride solution only 0·2 mg. An alternative technique is given in the A.O.A.C. handbook (1945).

ETCHING TEST. This well-known test has the advantage of being specific for fluorine, but it is not well adapted for quantitative work. Essery (1931) uses a lead block with a cavity 0·5 inch in diameter and 1 inch deep, into which is placed the substance to be tested. The fluorine must previously have been concentrated into a small bulk of inorganic material by precipitation and ashing. It is mixed in the lead cavity with a few drops of concentrated sulphuric acid, and a waxed microscope cover-slip on which a small pattern has been scratched placed over it. The block is then kept for several hours at 37° and the cover-slip examined for etching. Essery states that it is possible in this way to detect 2 mg. of fluorine in 100 g. of fruit pulp. Caley and Ferrer (1937) describe a similar method which permits of the reaction being carried out at 150° and claim to be able to detect 0·05 mg. of calcium fluoride after 30 minutes' heating. According to Hagen (1934), 0·005 mg. of fluorine can be detected by the 'oiliness' or 'greasiness' produced on the inner surface of a clean glass test-tube when the material is warmed with concentrated sulphuric acid.

A familiar modification is the 'hanging drop' test in which the substance under examination is mixed with silica or powdered glass and concentrated sulphuric acid, and the silicon fluoride which is evolved allowed to come into contact with a minute drop of water on the under side of a glass plate. A film of precipitated silica indicates the presence of fluorine in the sample.

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15

[BORON]

BORON is present in small quantities in all plants and appears to be necessary for their growth. It has not been shown to be an essential element in animal life. Its interest for the food chemist arises chiefly from the use of boric acid as a food preservative.

BORON AND PLANT GROWTH. In 1915 Mazé showed that boron was essential to the growth of maize but injurious in excess. Voelcker (1915), in pot experiments on wheat and barley, found that as little as 5 p.p.m. of boron in the soil had a slight toxic effect. Quantities of more than 10 p.p.m. prevented the plants from developing and forming grain. Neller and Morse (1921) found that a number of food plants growing in pots were injured by borax and concluded that 3 lb. of anhydrous borax per acre was the maximum amount tolerated by beans and 5 lb. per acre by maize and potatoes. These amounts would correspond to only 0·25 and 0·4 p.p.m. of boron in the top 12 inches of soil: later work has shown that they are in fact well below the optimum for root crops.

It has now been clearly established that although boron is highly toxic to plant life under certain conditions, it is an essential element in the growth of most plants (Warington 1923), and that boron deficiency in soils is responsible for several plant diseases. In general, the margin between deficiency and excess is narrow. It would seem that boron in traces has a certain definite function to perform in the metabolism of the plant and that anything above this small amount is injurious. Its action appears to be connected with the reproductive parts rather than the purely vegetative parts;

beans, for instance, will not set seed in absence of boron, and apples and pears develop internal cork in the fruit. Other symptoms of boron deficiency are brown heart of beet and other root crops, and chlorosis of leaves and growing points in various plants. Coloured illustrations of some of these conditions have been given by Wallace (1943, 1944).

Boron requirements and sensitivity to boron vary greatly with different plants and in different soils. As a rough approximation the optimum concentration of available boron in the soil for most plants is below 1 p.p.m. The liming of soil makes boron unavailable and the injurious effects of calcareous and limed soils on certain crops have been attributed partly to immobilisation of boron. Application of borax to such soils will often counteract the effects of overliming. According to Jones and Scarseth (1944) there is for each plant an optimum ratio between calcium and boron intakes.

For leguminous and leafy vegetables and potatoes the upper limit of available boron in the soil is of the order of 1 p.p.m. Root vegetables (beets, turnips, carrots, onions, etc.) require more. Beets are more susceptible to boron deficiency than any other vegetable. Cereals appear to be less dependent upon it, although stimulated in growth by small quantities. The usual dressing for root crops and potatoes when boron deficiency is suspected is 20 lb. of anhydrous borax per acre applied when preparing the land for sowing, but for cereals and grass land this is too high and would be followed by toxic effects. 20 lb. per acre would correspond to about 1·6 p.p.m. of boron in the top 12 inches of soil. Care is necessary in applying borax to land since its toxic effect may be cumulative and it is not readily eliminated from some soils.

Little is known of the function of boron in plants except that it appears to be connected with reproduction. Johnston (1928) suggested that it is one of the agents responsible for the translocation of starch and sugars in the plant. Without it, starch and sugars accumulate at the points where they are synthesised in the leaves and cannot be transported. The fact that boric acid combines with polyhydric alcohols and sugars may be significant in this connection. It is also possible that boric acid may be concerned in the maintenance of the vascular system of plants. Incomplete development of this system will interfere with the transport of starch and sugars. Quarendon (1938) points out that crops which suffer from boron deficiency in the field are usually those with extensive storage systems, the effect of the deficiency being to reduce the percentage of sugar contained therein.

Tauböck (1942) finds that boric acid gives with flavonols (quercitin, morin, myricetin, etc.) in presence of certain organic acids, yellow dyes which show a yellow-green fluorescence at high dilutions. The fluorescent product appears to be specific for flavonols and the reaction can be used to detect 0·01 μg. of boric acid. Tauböck claims that plants which contain flavonols have a relatively high boron content.

BORON NATURALLY OCCURRING IN FOOD. Boron, being an essential element in plant metabolism, is found in traces in most foods of vegetable origin. In general the smallest amounts are found in those plants such as wheat and barley which have the lowest boron requirements in soil, and the largest amounts in root crops and fruit which have relatively high requirements.

The boron content of foods is generally given in terms of boric acid, H_3BO_3, and in biochemical work usually in terms of boron. Some confusion may result from this, since 10 parts of boric acid are equivalent to only 1·8 parts of boron.

Figures for natural boric acid and boron in various foods, especially fresh and dried fruits, have been given by several authorities. Dodd (1929) found 0·003 to 0·006 per cent of boric acid (5 to 10 p.p.m. of boron) in fresh fruits and Allen and Tankard (1904) found 0·004 to 0·016 per cent (7 to 28 p.p.m. of boron). Oranges examined by Dunn and Bloxam (1929) contained 0·005 to 0·033 per cent of boric acid (9 to 59 p.p.m. of boron) in the peel and 0·002 to 0·008 per cent (3·5 to 14 p.p.m. of boron) in the pulp. It is a common practice to wash the skin of oranges with an antiseptic solution of boric acid before shipment, and one cannot be certain, with imported fruit, that the boric acid found is not partly due to such treatment.

Dodd (1929) found 0·01 to 0·022 per cent of boric acid (18 to 39 p.p.m. of boron) in currants, sultanas and raisins from the Mediterranean, Australia and California. In similar samples from Australia, stated not to have been treated in any way with boric acid, I have found amounts ranging from 0·016 to 0·027 per cent. These figures correspond approximately to 5–15 p.p.m. of boron in the fresh fruit and agree well with those found by other investigators for fresh fruit of different kinds. Brown (1936) gives similar figures.

In other dried fruits, Dodd found from 0·003 per cent of boric acid (5 p.p.m. of boron) in French and Californian prunes to 0·025 per cent (44 p.p.m. of boron) in Australian peaches. Some of these figures do not preclude the possibility that the fruit may at some stage have been washed with boric acid. It is clear, however, that a very small quantity of natural boron, when multiplied by 5½ to bring it to boric acid and again by 3 or 4 to represent concentration on drying, may become quite a considerable percentage of boric acid in the final product.

Cacao beans contain 0·022 to 0·084 per cent of boric acid, corresponding to 38–148 p.p.m. of boron (Dodd 1927) and chocolate roughly about one-fifth of these amounts. Seaweeds and edible products made from them may contain relatively large amounts, as much as 0·16 per cent having been found in agar-agar (Chapman and Linden 1926). Sea-water contains about 4 to 6 p.p.m. of boron, corresponding roughly to 25 p.p.m. of boric acid. It has been stated that salt mined in Italy is often highly contaminated with boric acid, sufficient to introduce from 0·004 to 0·020 per cent into some foods (Villavecchia and Barboni 1912).

Dairy produce and flesh foods contain the merest traces of boric acid. When ingested by animals in their food it appears to be rapidly and completely excreted. Milk does not normally contain more than 1 p.p.m. and eggs about 0·1 p.p.m. of boric acid (Bertrand and Agulhon 1913), but the administration of boron compounds to cows may result in a tenfold increase in the boric acid of the milk (Hove and others 1939). In Owen's experiments (1944) the addition of about 20 g. of borax daily to the ration resulted in an increase in the boron content of the milk from 0·7 to over 3·0 p.p.m.

The rennet used for cheese making often contains from 1 to 2 per cent of boric acid as a preservative, but most of this is separated in the whey. Even if the whole of it remained in the cheese it would not represent more than 0·005 to 0·01 per cent of the weight of the cheese.

BORIC ACID AS A FOOD PRESERVATIVE. It is not proposed to enter into a detailed discussion here of the arguments for and against the use of boric acid as a food preservative. Some of the remarks on pages 32 to 35 in connection with copper in foods are applicable also to preservatives. The use of boric acid in this country has been prohibited under the Preservatives Regulations of 1925–7, although more recently the prohibition has been temporarily relaxed for certain commodities (margarine and bacon) during the period of emergency (*Analyst*, 1940, 411, 413; 1941, 372). The Regulations of 1925 followed the recommendations of the Departmental Committee on the Use of Preservatives and Colouring Matters in Food (1924). This Committee in their report gave an outline of the medical evidence upon which their recommendations were based. This evidence, as far as it concerns boric acid, may be summarised as follows:

(i) *Effect on Normal Individuals.* Boric acid is excreted more slowly than it is absorbed and accumulates in the system. In doses of 7 to 15 gr. (0·5 to 1 g.) daily it interferes with the absorption of food, especially fats and nitrogenous substances, and there is a tendency to lose weight. Small doses of boric acid have been shown experimentally to irritate the alimentary canal. In certain circumstances it may produce albuminuria.

(ii) *Idiosyncrasy.* Certain people undoubtedly show idiosyncrasy towards boric acid, e.g. flatulence after eating boracised cream. Cases are on record in which nausea and vomiting have occurred after a single dose.

(iii) *Invalids.* In persons suffering from renal weakness the consumption of boric acid is probably not free from danger. It may have injurious effects on persons suffering from nephritis and possibly on other disorders.

(iv) *Masking of Incipient Putrefaction in Food.* Boric acid, by inhibiting the growth of organisms which are concerned in the decomposition of food, may favour careless and improper methods of food production and give an appearance of freshness to otherwise unsound food.

Several of these arguments apply to chemical preservatives as a class and not only to boric acid. The Departmental Committee recognised this and classified the commoner preservatives, according to their relative degrees of undesirability, in three groups :

1. Formaldehyde and hydrofluoric acid.
2. Boric acid and salicylic acid.
3. Benzoic acid and sulphurous acid.

They recommended that the first two groups should be prohibited and the last group admitted, but only for certain foods and in certain amounts.

The argument quoted by the Committee that boric acid may mask incipient decomposition in food otherwise unsound is to some extent borne out by the work of Levine (1921), who found that *Bacillus coli* grows slowly in 1 per cent peptone containing 0·63 per cent of boric acid while the closely associated organism *B. lactis aerogenes* dies off. Blair (1936) also found that a 0·5 per cent solution of boric acid in lactose peptone showed marked selective action for bacterial growths in 24 hours at 37°, and that many strains of bacteria, probably of faecal origin, are capable of growth while others are inhibited. This selective action is not peculiar to boric acid and applies more or less to other preservatives in suitable concentration. The principle is made use of in bacteriological practice for the separation of pathogenic from non-pathogenic organisms. Thus chemical preservatives in general, and boric acid in particular, may on occasions be a danger rather than a safeguard, in that they may inhibit the growth of organisms which cause commercial spoilage of food but may not have an equally effective action against specific food poisoning organisms.

In referring to the alleged cumulative effect of boric acid the Committee quoted evidence that a single dose is probably not completely excreted for five days, although half the amount taken may be excreted in 12 hours. Kent and McCance (1941) also find that 93 to 94 per cent of boric acid ingested with food is excreted in the urine within a week, which, they consider, demonstrates rapid absorption and metabolism. Clearly boric acid cannot be called cumulative in the sense that the term is applied to lead and arsenic, but only in the sense that, as considerable quantities are liable to be ingested daily in various preserved foods, the tissues are presumably never free from boric acid and its action is continuous.

The quantities of boric acid formerly introduced into food as a preservative were considerable. The maximum amount found in butter, margarine, bacon, etc., was about 35 gr. per lb., or 0·5 per cent, but this amount was rarely reached and the highest found in practice, in imported butter for instance, was about 0·25 per cent. In butter and margarine 0·5 per cent of boric acid would mean that the water present would be practically a saturated solution of the acid. Under the Milk and Cream Regulations, 1912, Amendment Order, 1917 (now withdrawn), the maximum amount of boric acid allowed to be added to cream was 0·4 per cent. The actual substance added was a neutral mixture of boric acid and borax.

Boric acid combines in equimolecular proportions with polyhydric alcohols and some sugars to form acid compounds such as glyceroboric, mannitoboric and levulosoboric acids. The reaction is made use of in determining boric acid by titration with alkali. According to Gilmour (1921) its antiseptic action is thereby increased, but it is not clear that this is so and it would seem that the object of adding glycerol or honey to boric acid in pharmaceutical preparations is to increase its solubility. If it is permissible to compare boric and sulphurous acids, combination with carbohydrates should diminish rather than increase the antiseptic action.

Coste and Garratt (1935) draw attention to the presence of boric acid in the enamel of hollow-ware and show that a ‘ soft ’ enamel may yield considerable amounts of boric acid if corroded and disintegrated by the action of hot solutions of organic acids.

THE DETERMINATION OF BORON IN FOOD

Methods for the determination of boron in food fall for the most part into four groups,

(i) Distillation as methyl borate and gravimetric determination in the distillate as calcium borate.
(ii) Titration with standard alkali after removal of phosphates and addition of a polyhydric alcohol, which combines with boric acid to form an acid complex.
(iii) Colorimetric determination with turmeric, quinalizarin or other reagents.
(iv) Spectrographic methods.

Both the titration and colorimetric methods may be combined with a methyl borate distillation.

DISTILLATION AS METHYL BORATE. The sample (10 to 20 g.) is ashed with the addition of sodium carbonate to retain boric acid, and the ash taken up with a little water and neutralised with nitric acid. A further 0·5 ml. of nitric acid is added and the whole made up to about 20 ml. This is introduced into a 250-ml. distilling flask carrying a dropping funnel and connected with a condenser, the end of which dips into a strong solution of ammonia. A small quantity of silver nitrate is added to the liquid in the flask to precipitate chlorides. The flask is heated in an oil or glycerine bath at 120° and methyl alcohol introduced from the funnel, drop by drop at first, then 1 or 2 ml.

at a time. When 15 ml. has been added the contents of the flask are distilled to dryness. The addition of methyl alcohol and distillation to dryness are repeated until a few drops of the distillate no longer give the turmeric reaction for boric acid. At this point 3 ml. of water are introduced into the flask and distilled to dryness. The ammoniacal distillate is added to a weighed amount of freshly ignited calcium oxide in a platinum dish and evaporated. The residue is dried at 160° and ignited to constant weight in a muffle furnace. The increase of weight due to formation of calcium metaborate CaB_2O_4 is calculated to boric acid.

In Robinson's method (1939) the material is ashed with the addition of barium hydroxide and the distillation carried out on a steam bath with phosphoric acid and methyl alcohol, the distillate being collected in normal sodium hydroxide solution. The boric acid in the distillate is determined colorimetrically (p. 393). In the A.O.A.C. method (1940) for boron in soils the sample (10 to 25 g.) is fused with three to four times its weight of sodium dihydrogen phosphate, the fused mass powdered and mixed with 100 ml. of phosphoric acid, 50 ml. of methyl alcohol added and the mixture steam-distilled into a small quantity of sodium hydroxide solution until about 500 ml. of distillate have been collected. The distillate is evaporated to dryness and the boric acid determined by titration after addition of mannitol.

Owen (1946) gives a continuous distillation method with phosphoric acid and methyl alcohol for the determination of traces of boron in plants, etc. The distillation flask is of fused silica, and the methyl borate is collected and hydrolysed in sodium hydroxide solution contained in a nickel receiver, to avoid contamination by boron extracted from glass. The boron is determined colorimetrically with quinalizarin (p. 393).

Alcock (1937) has also described a distillation method which gives excellent results. It is applicable to the amounts of boric acid which may be present as a preservative in foods, and is valuable as a check upon the results obtained by other methods. McHargue and Hodgkiss (1941) separate boron from soils by distillation with perchloric acid in presence of a fluoride. The boron distils over as fluoboric acid. The method is analogous to the determination of fluorine by distillation as hydrofluosilicic acid (p. 371).

DETERMINATION OF BORIC ACID BY TITRATION: THOMSON'S METHOD. When excess of a polyhydric alcohol such as glycerol, mannitol or invert sugar is added to a solution of boric acid the compound formed is sufficiently acid to be titrated with standard sodium hydroxide and phenol-phthalein, the end-point corresponding to the formation of sodium metaborate $NaBO_2$. If phosphates are present they interfere with the titration and make it impossible to obtain a sharp end-point, probably owing to the formation of glycerol or mannitol phosphoric acids. Thomson (1895) therefore added to the solution enough calcium hydroxide to precipitate all the phosphate while leaving boric acid in solution and determined the boric acid in the filtrate. As applied to the determination of added boric acid in milk his method is as follows.

100 ml. of milk are evaporated to dryness on the steam bath in a platinum dish with 1 to 2 g. of sodium hydroxide. The residue is thoroughly charred at a low temperature and completely extracted with dilute hydrochloric acid. The extract is transferred to a 100-ml. flask, the bulk not being allowed to exceed 50 to 60 ml., 0·5 g. of dry calcium chloride added, then a small quantity of phenol-phthalein solution and finally 10 per cent sodium hydroxide solution until a permanent slight pink colour is perceptible. 25 ml. of lime water are then run in and the liquid made up to the mark. All the phosphoric acid should now have been precipitated as tribasic calcium phosphate, leaving boric acid in solution. The mixture is filtered through a dry paper and 50 ml. of the filtrate taken for titration. A small quantity of methyl orange is added and the liquid exactly neutralised to methyl orange (yellow tinge) with dilute sulphuric acid and N/5 or N/10 sodium hydroxide. At this stage the liquid contains the strong acids combined as salts, only boric and carbonic acids existing in the free state. Carbonic acid is expelled

by boiling for a few minutes, the solution cooled, a little more phenol-phthalein added and then 30 g. of glycerol (previously neutralised to phenol-phthalein). The liquid is now titrated with N/5 or N/10 sodium hydroxide solution until neutral to phenol-phthalein. 1 ml. of N/10 sodium hydroxide solution is equivalent to 0·0062 g. of boric acid.

This method, which for a long time remained the standard method for boric acid in foods, has been criticised and modified in several particulars. Firstly it has been found difficult to avoid precipitating boric acid as calcium borate. In the Government Laboratory method (*Analyst*, 1923, **48**, 416) it is emphasised that after adding calcium chloride to the solution the addition of sodium hydroxide must be done very carefully drop by drop with constant stirring to avoid too high local alkalinity, and that the solution must not be made more than faintly alkaline to phenol-phthalein. Lime water should not be added at this stage or calcium borate may be precipitated. Deerns (1922) claims that the interference of phosphates may be obviated by adding ferric chloride to the hydrochloric acid solution of the ash followed by neutralisation with calcium carbonate. The liquid is filtered and boric acid determined in the filtrate by titration with sodium hydroxide and mannitol. Kolthoff (1922) finds that if the acid solution of the ash containing phosphoric acid is neutralised to phenol-phthalein with sodium hydroxide in presence of excess of sodium citrate, addition of mannitol makes it possible for boric acid to be titrated without interference from phosphoric acid (cf. the use of citrate in the determination of lead in presence of calcium phosphate).

An alternative method of overcoming the difficulties caused by the presence of phosphates is to remove them by precipitation with magnesia mixture (Monier-Williams 1923). From the alkaline solution of the ash the greater part of the calcium is first separated by acidifying with sulphuric acid and filtering. In this way precipitation of calcium borate is avoided when the liquid is subsequently made alkaline. Phosphate is now precipitated by addition of magnesia mixture and removed by filtration, and boric acid is determined in the usual way with glycerol or mannitol in the filtrate. Methyl red is used as an indicator in preference to methyl orange since the colour change is much sharper. In the A.O.A.C. method (1940) for boron in soil bromthymol blue is used as indicator, as being less affected by carbonic acid.

A second important criticism of the Thomson method is that it is impossible to carbonise foods containing much fat or oil, even after addition of alkali, without loss of boric acid. Dodd (1929) has examined this point particularly and has shown that the presence of much fat apparently causes the volatilisation of boric acid as glyceroboric acid. He recommends that the fat should be extracted with light petroleum, and any boric acid taken up by the solvent removed by shaking it with sodium hydroxide solution. The sodium hydroxide extract is then added to the main fat-free sample and the whole carbonised as in Thomson's method. The Government Laboratory method already referred to is similar except that ethyl ether is used for extracting the fat.

It would seem that most workers who have investigated this point have used too little alkali. If sufficient sodium hydroxide is added, at least 2 g. for every 10 g. of fat, there is no loss of boric acid. 10 g. of butter to which have been added 2 g. of sodium hydroxide dissolved in the least possible amount of water, can be burnt off in a platinum dish over a free flame and the carbonisation completed in a muffle furnace at a low heat without any loss of boric acid. In Dodd's experiments the highest ratio of alkali to fat was 0·12 g. to 1 g. of fat, and with this there was a serious loss of boric acid. In my experience double this quantity of alkali for each gramme of fat prevents any such loss. Probably it is necessary that enough sodium hydroxide should be present to ensure complete saponification of the fat as well as to combine with all the boric acid ; otherwise all the alkali will be neutralised by organic acids formed on combustion and boric acid will be liberated. The method given above (Monier-Williams 1923), with the addition of enough sodium hydroxide to prevent loss of boric acid during carbonisation of fat, has been applied successfully to a variety of foods and obviates the troublesome extraction with fat solvents. It can be used for the determination of boron occurring naturally in dried fruit by ashing 100 g. of material with 5 g. of sodium hydroxide, and evaporating the final filtrate to a convenient volume for titration, but for still smaller traces of boron in many plant products it may be necessary to use colorimetric methods.

A further point in connection with Thomson's original method concerns the removal of carbonic acid from the solution by boiling before the titration. In the Government Laboratory method (1923) boiling is continued for at least 10 minutes, otherwise there is a danger of high results from residual carbonic acid. Such boiling does not cause loss of boric acid (Dodd 1929). This would seem to apply only to boiling in a vessel covered with a clock-glass to prevent loss by spirting, since Brown (1936) finds that in an open conical flask boiling for one minute is enough.

The Thomson method as applied to small quantities of boron in dried fruit has been thoroughly investigated by Brown (1936). The question of loss of boric acid in the carbonisation of fat does not arise with dried fruits, but a number of other precautions necessary in order to obtain trustworthy results are discussed by him. He also describes an electrometric titration method for boric acid.

Germanic acid resembles boric acid in forming acid compounds with polyhydric alcohols, but is of rare occurrence (p. 494).

COLORIMETRIC METHODS. The well-known reaction of boric acid when heated with an extract of turmeric has been used by a number of analysts for its approximate determination. Cassal and Gerrans in 1902 found that the delicacy of the colour reaction was increased by the addition of oxalic acid, and Cribb and Arnaud (1906) prepared turmeric test papers containing oxalic acid which would detect as little as 25 p.p.m. of boric acid in solution, or 2·5 mg. in 100 ml. By progressive dilution of the liquid the point can be found at which it gives only a faint pink colour with the paper, and the approximate boric acid content thus ascertained.

In recent years the turmeric method has been greatly improved and Robinson (1939) has shown that in conjunction with a methyl borate distillation it is capable of giving very accurate results with the small quantities of boric acid which occur naturally in plants. In his method acid-washed silver sand is coated with the yellow colouring matter of turmeric by evaporation of an ether extract. A weighed quantity of the turmeric-coated sand is treated with a solution containing the boric acid to be determined, together with oxalic acid, and the mixture evaporated to dryness. When the reaction between the turmeric and boric acid is complete the sand is extracted with 70 per cent alcohol until colourless and the intensity of the red colour component in the solution measured in a Lovibond tintometer. Standards are prepared with known amounts of boric acid and the Lovibond units plotted against milligrams of boric acid on a graph. Since the relation between the number of red units and the amount of boric acid is strictly linear a graph is hardly necessary. Each red unit is equivalent to 0·005 mg. of B_2O_3 in 100 ml. of solution. The method is particularly suitable for amounts of boric acid ranging from 0·01 to 0·1 mg.

Hydroxy-anthraquinones, particularly quinalizarin, which are intensely coloured in concentrated sulphuric acid, show with traces of boric acid a marked change of colour. When used as a micro-test with a drop of solution and two or three drops of sulphuric acid containing quinalizarin the reaction will show 0·06 μg. of boron. Smith (1935) has adapted this test to the examination of alloys and minerals, and Berger and Truog (1939) and McHargue (1941) have applied it to the determination of boron in plant ash (cf. also Scharrer and Gottschall 1935). The colour change with boric acid is from reddish violet to blue, and the greatest sensitivity is in 93 per cent sulphuric acid. The solution of the ash of the sample must therefore be brought to this strength of acid by addition of more concentrated acid. Fluorine interferes with the colour change but the amounts naturally present in plants are too small to have any effect. Germanium also gives a colour change similar to, but much less intense than, that of boron; it is rarely met with in sufficient quantity to cause appreciable errors. Full details of the method are given in Smith's paper.

Dickinson (1943) has modified the quinalizarin method by the use of standards made from a mixture of phenolphthalein and thymol-phthalein, which obviates the necessity of making up fresh boric acid standards daily. He has also given a new colorimetric method using alizarin-S, the colour being matched with different amounts of methyl-orange solution. The reaction must be carried out in concentrated sulphuric acid. A variation of ±1 per cent in the acid concentration will cause an error of ±6 per cent in the amount of boric acid found.

Owen (1946) maintains that differences in the intensity of the colour with quinalizarin are due not so much to water content as to varying solubility of quinalizarin in the acid.

SPECTROGRAPHIC METHODS. Kent and McCance (1941) determine boron in biological material by the method already referred to under lead (p. 97), using iron as an internal standard. Foster and Horton (1937) have also described a spectrographic method combining the use of the Morton wedge photometer.

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[SILICON]

SILICA, as is well known, gives rise to a serious condition (silicosis) when inhaled as dust, owing to local action on the lung tissue, but when ingested with food it appears to be more or less inert. A series of investigations into the biochemistry of silica has been carried out by King and his co-workers (1933–9). They find that when taken into the digestive tract it is very largely eliminated in the faeces, but a considerable amount is dissolved by the alkaline duodenal juice and passes into the blood stream, from which it is rapidly eliminated by the kidneys without being retained by any of the organs of the body. Elimination in the urine takes place so quickly that the silica content of the blood, normally about 10 p.p.m., hardly rises at all, while that of the urine may increase greatly. Silica is possibly also excreted from the blood into the large intestine. Urinary silica varies normally from about 3 to 8 p.p.m. in carnivorous animals (cats) to 100–300 p.p.m. in herbivorous animals (rabbits). In human beings it is about the same as that of the blood, 10 p.p.m. or about 12 mg. in 24 hours (Whitehouse 1937), but shows wide fluctuations even on a constant diet (Goldwater 1936).

Silica occurs in gelatinous form (as a constituent of plant tissues), in amorphous form (diatom skeletons, flint dust, etc.) or crystalline. All of these are to some extent soluble in the alkaline digestive juices, the rate of solution depending mainly upon fineness of division. Flint or quartz reduced by grinding to a particle size of the order of the dust in the air, i.e. 1 to 10 microns, is freely dissolved and absorbed from the digestive tract and eliminated in the urine. Some complex silicate dusts are partially hydrolysed by the hydrochloric acid of the stomach, and the silica subsequently absorbed from the small intestine (King and McGeorge 1938). According to Riesser and Kindt (1928) when 1·3 g. of silica is ingested daily in food as much as 34 per cent of it may be absorbed and excreted in the urine. Holzapfel and Kerner-Esser (1943) find that much of the silicon in blood is soluble in alcohol and suggest that it is present in organic combination.

SILICA IN FOODS. Vegetable foods are the chief source of dietary silica. In these the silica content may vary from about 1 per cent of the ash in yeast up to 60 per cent, or more, of the ash in wheat, bran and other fibrous products. Dairy products and flesh foods contain much less. Strohecker, Vaubel and Breitwieser (1935) found the normal silica content of milk to be less than 1 p.p.m. but that of condensed milk and milk powder corresponded to 3·5–4·0 p.p.m. in the original milk, which indicated an accession of silica during processing. It is possible, however, that the method they used (colorimetric as yellow silicomolybdate) was not sufficiently delicate to determine accurately the small amounts present in 50 ml. of milk. In meat they found from 5 to 15 p.p.m. of silica and 30 to 40 p.p.m. in liver, but the variations were considerable. Eggs contain normally from 20 to 40 p.p.m. in the white and yolk. When they are preserved in a solution of water-glass (sodium silicate) no increase of silica takes place in the substance of the eggs although the alkalinity rises. The shell, on the other hand, which contains normally about 60 to 90 p.p.m. of silica, may show up to 0·3 per cent after 24 days of water-glass storage.

Beer is often high in silica, presumably extracted from the grain, and its consumption greatly increases the silica content of the urine (Whitehouse 1937). Gelatinous silica has been used for fining beer in place of isinglass, and this may add another 100 p.p.m. to the beer.

The presence of gelatinous silica in food appears to have no dietary significance. Rabbits have been given 2 g. of silica gel daily for 30 weeks without any ill effects (Gye and Purdy 1924). In therapeutics magnesium silicate is frequently given in large doses in place of the sulphate. It is questionable whether crystalline or amorphous silica may not act as an irritant. This probably depends largely on the size of the particles. Talc (magnesium hydrogen silicate) used for the facing of rice and sometimes in medicinal tablets, has been alleged to have an irritant action, and this would certainly be true of glass powder. The addition of silica dust to wheat and other grain as an insecticide is also viewed with suspicion. Possibly this is due not so much to the likelihood of its getting into the flour, since the grain would normally be cleaned before milling, as to the danger of silicosis among workmen in grain stores.

THE DETERMINATION OF SILICON IN FOOD

GRAVIMETRIC METHODS. For the gravimetric determination of silica in foods and biological material, organic matter is oxidised with nitric and sulphuric acids, the acid fumed off in platinum and the residue moistened with a few drops of water and ignited. A small quantity of hydrochloric acid is added and evaporated to dryness, and the residue heated at about 140° for an hour, this treatment being repeated twice to dehydrate the silica. The residue is boiled with water and the solution filtered through a close paper. The filter-paper is ashed in platinum and the residue ignited and weighed. It is then treated with sulphuric and hydrofluoric acids until no further loss of weight takes place on ignition. The difference between the two weighings represents silica (Kindt 1928). If a differentiation between silica of constitution and adventitious sand is required the sulphated ash must be boiled repeatedly with a saturated solution of sodium carbonate containing a small quantity of sodium hydroxide. The insoluble residue is washed with hot water and then with dilute hydrochloric acid and again with water until free from chlorides. It is dried, ignited and weighed as sand (A.O.A.C. handbook 1945).

King (1939) uses the following method for tissues high in silica. An amount of the dry powdered tissue is taken which may be expected to yield 5 to 50 mg. of silica. It is mixed with four times its weight of anhydrous sodium carbonate in a platinum crucible and heated until a clear liquid melt is obtained. The melt is dissolved in water and the solution evaporated with hydrochloric acid to dryness on the steam bath after which the residue is heated for 1 hour at 105°. The evaporation with hydrochloric acid and heating are repeated. The residue is taken up with dilute hydrochloric acid and the solution filtered through a close filter or by means of a ' filter stick ', which permits of filtration without the necessity of transferring the solution to a second vessel. The silica is washed with dilute hydrochloric acid, dried, ignited in platinum and weighed. It is then volatilised by treatment with sulphuric and hydrofluoric acids and the crucible reweighed. Alternatively the silica in the ash may be dehydrated by heating with 70 per cent perchloric acid (b.pt. 203°) which is a powerful dehydrating agent (Morgan and King 1932).

Davidson (1931) points out that silica in straw ash retains soluble bases obstinately and that volatilisation with hydrofluoric acid is essential in order to obtain accurate results both for silica and for bases.

COLORIMETRIC METHODS. Silicic acid combines with molybdic acid to give a yellow silicomolybdic acid complex, the colour of which can be compared with standards (Diénert and Wandenbulcke 1923). This complex on reduction gives a blue compound which is more suitable than the yellow compound for colorimetric measurement and is much more sensitive to small quantities of silica. Of the two methods the one depending on reduction to the blue colour is the more readily interfered with by phosphates, arsenates and iron, all of which must therefore be removed from the solution (cf. p. 198).

Reduction of the yellow silicomolybdic acid complex to the blue compound has been effected with a variety of reducing agents by different workers. Sodium sulphite solution at boiling-point, stannous chloride, sodium thiosulphate, quinol, benzidine and pyrrol have all been used at various times. The most satisfactory of all is a solution of 1:2:4-aminonaphthol sulphonic acid in sodium bisulphite.

For the analysis of foods or biological material King (1939) gives the following method. 0·1 to 0·5 g. of the dry material (depending on its silica content) is fused in a platinum crucible with sodium carbonate until a clear melt is obtained. This is dissolved in water and the solution neutralised to Congo red with dilute sulphuric acid. Ferric chloride and sodium acetate are now added and the solution diluted and boiled. This process precipitates the whole of the iron and phosphate as ferric phosphate and basic ferric acetate. An aliquot part of the clear filtrate, containing 0·2 to 1 mg. of silica, is introduced into a 50-ml. glass cylinder and treated with 4 ml. of a 5 per cent solution of ammonium molybdate in normal sulphuric acid. The yellow colour is compared with that of standards, using a solution of sodium silicofluoride containing 1 mg. of SiO_2 in 10 ml. The presence of hydrofluoric acid does not interfere with the development of the colour as it does with arseno-molybdate (p. 201). Diénert and Wandenbulcke (1923) used picric acid solution as a standard, 0·0367 g. of picric acid per litre giving a colour equivalent to that given by 0·050 g. of silica per litre. Swank and Mellon (1934) use a buffered solution of potassium chromate.

For amounts of silica too small for convenient determination by the yellow colour method, the blue colour method is used. A quantity of the filtrate which may be expected to contain from 0·02 to 0·12 mg. of silica is transferred to a 25-ml. flask and treated with 20 ml. of water, 2 ml. of acid molybdate solution and 0·5 ml. of a 0·2 per cent solution of 1:2:4-aminonaphthol sulphonic acid in sodium sulphite solution. The flask is shaken and allowed to stand for 10 minutes. 1 ml. of 10N sulphuric acid is added to stabilise the blue colour, and the contents of the flask are made up to the mark and mixed. The colour is then compared with that of standards similarly treated. Fuller details of these methods are given in King's paper (1939). He also gives details of a method in which silica is completely precipitated from solution as the pyramidon salt of silicomolybdic acid. It can thus be concentrated into a small volume and a more intense blue colour obtained on reduction with aminonaphthol sulphonic acid.

In all silica determinations care must be taken not to allow alkaline solutions to come into contact with glass vessels or silica will be taken up. King uses vessels made of a non-silicious urea-formaldehyde resin.

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17

[ALUMINIUM]

IN PLANT AND ANIMAL TISSUES. Aluminium, although representing, in its various combinations, as much as 7 to 8 per cent of the earth's crust, is almost completely absent from plant and animal tissues. The aluminium found in plants is nearly all due to accidental contamination with earth and dust, and the more thoroughly the plants are cleaned, even to the extent of scrubbing the harder parts with soap and water, the less is the amount of aluminium found in them. Figures showing considerable amounts of aluminium in stems and leaves of plants are generally the result of insufficient removal of extraneous dust, and the true aluminium content of such tissues is rarely more than 5 to 10 p.p.m.

Many water plants and plants growing in wet acid soils take up a certain amount of aluminium, but the metal is confined to their roots and does not penetrate to the stems or leaves. There are a few exceptions, the most notable of which are the club-mosses and sphagnum mosses. The ash of the common club-moss, *Lycopodium clavatum*, contains as much as 30 per cent of aluminium. This is present in the plant as a soluble organic salt which can be extracted with water. Several species of plants, of different genera, mostly from tropical regions, have been found to be aluminium accumulators and to contain relatively large amounts of aluminium in the leaves (Chenery 1946).

Hydrangea flowers are turned from pink to blue by increasing the amount of soluble aluminium in the soil, owing to the formation of a blue aluminium-delphinidin complex (Chenery 1937, Allen 1943). A similar change of colour from red to blue can be induced in the shoots of red cabbage when this is grown on media containing soluble aluminium (Kratzmann 1914). For some plants growing on acid soils aluminium may be essential, but in general it would seem that the membranes of root cells are not completely impervious to aluminium in solution and that the plant, being unable to exclude it altogether, becomes inured to its presence. If the concentration of aluminium in the soil solution rises above 0·03 per cent all plants, even those which prefer an acid soil, are killed. The soluble aluminium of acid soils may be one of the chief causes of their infertility (Burgess 1923, Conner and Sears 1922). Addition of phosphates to such soils reduces the amount of soluble aluminium by precipitating aluminium phosphate (Eisenmenger 1935). Iron and manganese in solution are likewise toxic to plants under much the same conditions as aluminium. In fertile soils aluminium, iron and manganese appear to exist almost wholly in insoluble forms (cf. pp. 248 and 301).

Human and animal tissues, with the exception of the lungs and possibly the liver (p. 408), contain even less aluminium than plants. Myer's investigations in 1928 showed that practically none of the organs of the body contain naturally more than one or two parts per million. Blood is stated to contain about 0·7 p.p.m.

Aluminium is practically non-existent in sea-water ; only 0·5 p.p.m. (Haedler and Thompson 1939).

ALUMINIUM IN FOOD. The presence of notable amounts of aluminium in food may be due to (i) sodium aluminium sulphate baking powders, (ii) aluminium vessels and cooking utensils, (iii) aluminium salts added occasionally to canned foods (crustacea and shell-fish) to prevent discoloration (Fellers and Harris 1939), (iv) alum added to preserved and pickled fruits and vegetables, e.g. pickled cucumber and maraschino cherries, to make them harder and crisper, and (v) alum added as a bleaching and improving agent in flour and bread. The two last-named uses of alum are apparently obsolete, as no instances have been reported in recent years. Improved methods of pickling and preserving fruit and vegetables have probably nullified any supposed advantages which may have been obtained from alum treatment, and the technique of flour ' improving ' has long since advanced beyond the alum stage.

ALUM BAKING POWDERS. The use of sodium aluminium sulphate as the acid constituent in the so-called ' alum ' baking powders is also practically obsolete in this country. It was discontinued about forty or fifty years ago as a result of action by local authorities. In the United States alum baking powders are used almost as widely as phosphate powders, and at one time there was considerable rivalry between the commercial interests controlling the two products. Each of these interests appears to have spent large sums of money on experiments and on propaganda, in trying to persuade the public on the one hand that aluminium salts are perfectly harmless and on the other that aluminium in all forms is toxic, not only in baking powder but even in the small amounts dissolved by food from aluminium cooking vessels.

The acid constituent of alum baking powders is anhydrous sodium aluminium sulphate, $Na_2SO_4,Al_2(SO_4)_3$, which reacts with sodium bicarbonate giving sodium sulphate, aluminium hydroxide and carbonic acid gas. Its action on bicarbonate is slower than that of acid calcium phosphate, and the evolution of gas in the dough takes place more gradually. So-called combination baking powders are much used in the United States ; they contain both sodium aluminium sulphate and acid calcium phosphate and combine the advantages of a fairly quick initial production of gas in the dough and of a slow subsequent action maintained for a longer time.

METALLIC ALUMINIUM IN FOOD INDUSTRIES. Aluminium is widely used for food manufacturing plant, especially in dairies and in breweries, for household cooking vessels, and more recently as an alternative to tin-plate in food canning, particularly for fish packed in oil ; also as a wrapping foil for butter and other foods (Hood and White 1939). It is rather easily corroded by certain foods and thus finds its way into the food either as a soluble salt or as aluminium hydroxide. Aluminium ware has therefore not escaped the criticism directed at the use of alum in foods.

Normally the amount dissolved by food is very small, owing mainly to the inhibiting effect of colloids. Friend and Vallance (1922) found that in presence of only 0·1 per cent of gelatin the rate of corrosion of aluminium in 3 per cent salt solution was reduced by half. A similar effect is produced by colloids on the corrosion of iron, zinc, lead and other metals and is due apparently to adsorption of the colloid on the surface of the metal.

Apart from the effect of colloids the resistance of aluminium to corrosion depends largely on the purity of the metal. Aluminium of 99·5 per cent purity is far more resistant than 94–95 per cent metal. Metal of 99·9 per cent purity is still more resistant (Reif and Steinbeck 1937) and even purer grades can now be produced. Aluminium alloys are in general more easily corroded by acid foods, except those with magnesium, manganese and chromium.

Bryan (1936, 1937) has investigated the action of foods upon aluminium, chiefly from the view-point of its use as a substitute for tin-plate in canning. Corrosion of both tin and aluminium may be of two kinds, the oxidative and the hydrogen evolution types. Corrosion of tin is largely of the oxidative type while that of aluminium is characterised by hydrogen evolution. At ordinary temperatures the oxide film on aluminium withstands attack by citric acid fairly well unless salt is present, when corrosion is greatly increased. Similarly salt alone, even in hot solutions, corrodes aluminium only slightly when no acid is present, but quickly in presence of organic acids. Corrosion by acid brines is accelerated by oxygen (Bryan and Morris 1940). In canned fruit the resistance of the oxide film to solution by citric acid is not complete, and corrosion takes place gradually with formation of hydrogen swells. Aluminium cannot therefore be used for these products unless double-lacquered. A high pressure of hydrogen in the can is seldom reached owing to the permeability of the metal to hydrogen.

It has been found that if organic acids are neutralised so as to bring the pH of the solution to the neighbourhood of 7·0, a striking difference is observed between the corrosive action of hydroxy- and of non-hydroxy acids. Solutions of the sodium salts of hydroxy-acids, such as citric and tartaric, corrode aluminium readily while those of non-hydroxy acids, such as acetic, succinic and benzoic, have no effect whatever. This is due, apparently, to the formation by aluminium hydroxide of soluble basic complexes with the anions of hydroxy organic acids (Bryan 1936, 1937, Morris and Bryan 1936).

For canning fish, such as sardines in oil and shell-fish, aluminium has been used with success. It is preferable to tin-plate in that it rarely imparts any taste or smell to the food nor does it cause blackening like that due to iron sulphide. The cans are light and easily embossed, thus making it possible to do without labels. At the same time aluminium is not so strong as tin-plate, is more difficult to make into cans and is more expensive. According to Lunde and others (1937) storage of fish and other food in aluminium containers for several years may raise the aluminium content of the food to about 100 p.p.m. Aluminium has also been used successfully for canning milk, meat and vegetables. The presence of fat or oil seems to protect it from corrosion.

Since aluminium forms only one series of salts it cannot act as an oxidation catalyst in food or hasten the onset of rancidity in oils and fats. It may cause turbidity in wines by precipitating tannins and is said to resemble iron in giving a metallic taste to beer (Schreder and others 1937). According to Sylvester (1935) it has a bad effect on the colour of tea and coffee, probably owing to the formation of coloured lakes with the tannins present.

A number of processes have been patented for producing durable films upon aluminium, both chemically and electrolytically (Neurath and Einerl 1941). Aluminium treated by these processes is more resistant than the untreated metal to corrosion by acid foods (Reif and Steinbeck 1937).

CORROSION OF ALUMINIUM COOKING VESSELS. Aluminium is not attacked by distilled water, whether hot or cold, but hard water corrodes it slightly and may become cloudy with suspended aluminium hydroxide. London tap-water boiled in aluminium saucepans and allowed to stand overnight takes up from 8 to 20 p.p.m. of the metal. Water softened by base exchange processes, using zeolites regenerated with salt, may be extremely corrosive to aluminium, and kettles in which such water is used have a comparatively short life (cf. Derby 1935).

Lactic acid is not so corrosive to aluminium as might be expected and sour milk has only a slight action. Fruit cooked in aluminium takes up about 9 to 12 p.p.m. A mutton broth containing mixed vegetables cooked in an aluminium saucepan took up 6 p.p.m., increasing to 10 p.p.m. when the broth was allowed to stand in the pan for 24 hours after being cooked. The metal is attacked more readily by weak alkalies than by weak organic acids, and vegetables cooked with sodium carbonate may show as much as 90 p.p.m.

The black stain often seen on aluminium vessels after use has been stated by various authorities to be due to (i) silicon (Formenti 1905), (ii) iron (Tinkler and Masters 1924), (iii) an optical effect due to a very finely etched surface (Fischer and Geller 1938). Possibly all three factors may be concerned according to the particular conditions.

ALUMINIUM AND HEALTH. Aluminium salts are astringent and resemble iron salts in this respect. Their action when ingested must be considered from two points of view, (i) the absorption of aluminium into the blood stream and its possible action on the blood or other organs of the body, and (ii) the local effect of aluminium salts upon the lining of the stomach and intestines and possible interference with digestion.

Absorption and Excretion of Aluminium. The solubility of aluminium hydroxide in the contents of the alimentary tract seems to depend upon the amount and nature of the food. Actual experiments carried out on human subjects and on dogs, by withdrawing the stomach contents after ingestion of food prepared with alum baking powder, have shown that a variable proportion, averaging perhaps one-third of the total aluminium, is present in solution. Salts of aluminium are precipitated by proteins in weakly acid solutions (Maillard and Wünschendorff 1926) so that in presence of much food, aluminium may to some extent be prevented from going into solution. In the small intestine, where most of the absorption of food constituents takes place, one would expect the solubility to be greater, owing to the alkaline character of the medium ; Myers and Killian (1928), however, found it to be less than in the stomach and to bear little relation to the acidity or alkalinity of the gastric and intestinal contents.

Determinations of aluminium in blood and in urine after ingestion of aluminium salts show that the metal is absorbed with difficulty and that only very small amounts diffuse into the blood from the alimentary tract. Absolute impermeability of the epithelial membranes is hardly to be expected, and after large doses one or two parts per million can be detected in the blood (Judd Lewis 1931, Underhill and Peterman 1929). Diffusion is promoted by several agents such as bile salts, urea, etc. On the whole it would seem that the absorption of aluminium from the alimentary tract takes place much less readily even than that of iron.

No indications of anaemia or other effects on the blood have been observed in experimental aluminium feeding either with animals or with human subjects. Aluminium which gains access to the blood after large doses does not appear to be excreted in the urine or to accumulate in the tissues of the body. Possibly it is re-excreted into the small intestine, but the amounts in question are so small that they are not easy to trace analytically.

The only organs of the body in which appreciable quantities of aluminium have been found are the lungs and liver. Underhill and Peterman (1929) found over 50 p.p.m. in the lungs of a dog aged 15 years, presumably an accumulation of aluminium silicate entering in the form of dust. Lundegårdh (1934), using a spectrographic method, found an average of 30 p.p.m. in human livers from hospital patients.

While no toxic effects have been observed from the absorption of traces of aluminium, it must be remembered that this metal readily forms compounds with organic substances, as instanced by the formation of lakes with many colouring matters, and that its presence in the blood even in very small amount may conceivably have some significance. Flinn and Inouye (1928) have discussed this question and have suggested that even metals considered to be non-toxic may possibly combine with constituents of the blood cells or serum and affect some of their functions, such as those of resistance to disease, although no apparent action can be detected. It is almost impossible to put these suggestions to actual test but if there is any substance in them they would constitute an argument for keeping extraneous metals out of food as far as possible, and not placing too much reliance on negative evidence as to their effects on health. Apart from these speculative suggestions there is no trustworthy evidence, or even reasonable suspicion, that aluminium in food, in amounts such as are derived from vessels or appliances in commercial or domestic use, has any adverse effect on health.

Action of Aluminium Salts on the Digestive Tract. As ordinarily used, baking powders prepared with sodium aluminium sulphate may introduce into bread about 0·05 per cent of aluminium, or 500 p.p.m. This has been held by many to interfere seriously with digestion, although no conclusive evidence of it has been adduced. An American referee board in 1908 carried out a long series of experiments upon squads of volunteers, who received daily doses of aluminium in this form ranging from 0·06 to 0·97 g. The larger doses produced gastro-intestinal irritation and colic but the smaller doses, such as would normally be consumed in bread, had no obvious effect. Whether the action of large doses was due primarily to the aluminium compound or to sodium sulphate in the baking powder residue was not clear. It would seem that the effect of the aluminium, if any, may have been obscured by the cathartic action of the accompanying sodium sulphate. Experiments by Wührer (1934) and by Schaeffer (1928) have indicated that large doses of aluminium salts may cause gastric discomfort while the effect of smaller doses is not noticeable.

Colloidal aluminium hydroxide has been widely used to neutralise acidity in cases of gastric and duodenal ulcer. In addition to the neutralising or adsorbing action of the hydroxide it is possible that the astringency of the resulting soluble salts of aluminium may have some effect in checking the flow of gastric juice.

Aluminium may combine with phosphoric acid and make it insoluble and unavailable in the small intestine. Leary and Sheib (1917) found that this occurred to some extent in puppies and rats receiving 0·1 to 0·3 g. of aluminium daily in their food. In some of the experiments carried out for the United States referee board, indications were obtained that aluminium caused a decrease in urinary phosphorus and a corresponding increase in faecal phosphorus. The effect on the phosphorus balance of the body was, however, negligible. It has been stated that very large doses of aluminium salts given experimentally in excess of the total phosphorus intake may produce rickets, the action being similar to that of beryllium (Cox and others 1931, Deobald and Elvehjem 1935, Street 1942).

It would seem that the evidence available on the effect of large doses of soluble aluminium salts upon digestion and assimilation, while not conclusive, is sufficient to justify the suspicion attached to the use of alum baking powders. At any rate there is, in this country, a widespread aversion to their use which is undoubtedly based on sound principles, and the general opinion among food chemists is that to admit alum baking powders would be an unnecessary and retrograde step.

THE DETERMINATION OF ALUMINIUM IN FOOD

DESTRUCTION OF ORGANIC MATTER. There is no loss of aluminium on dry ashing at moderate temperatures, but if the temperature is too high aluminium oxide becomes difficult to dissolve in hydrochloric acid. Eveleth and Myers (1936) prefer dry ashing to wet oxidation. Satisfactory results are obtained by working on the sulphated ash, for which the material is charred in a silica dish, and the char moistened with sulphuric acid and ignited in a muffle furnace at a low heat. Lampitt and Sylvester (1932) use a wet-oxidation method with sulphuric and nitric acids in a silica flask. A small point arises here if traces of aluminium are being looked for. It is generally found that there is a slight residue insoluble in strong acids. This residue is often neglected as being due to sand or dust in the sample. It may contain aluminium silicate derived from soil and if it is desired to ascertain the total amount of aluminium in the sample, irrespective of origin and state of combination, this insoluble residue must be fused with alkali and the resulting solution added to the main portion. Whether it should be included in the total depends upon the nature of the material and the purpose for which the analysis is required.

SEPARATION FROM IRON. As with several other metals occurring in food and biological material an important step in the determination of aluminium is its separation from iron. If no iron is present it is generally necessary to add some, in order to facilitate the initial precipitation of aluminium. The methods for this separation fall into four groups.

(i) Solution of aluminium hydroxide, phosphate or basic acetate in caustic alkali, in which the corresponding iron compounds are insoluble. This is a general method of separating aluminium from iron (cf. Lampitt and Sylvester 1932).

(ii) Separation of iron by special methods such as—

(*a*) precipitation with ‘ cupferron ’, the ammonium salt of nitroso-phenylhydroxylamine (Lundell and Knowles 1929, Hart 1932, Cholak and others 1943) ;

(*b*) conversion into thiocyanate and extraction with ether (Underhill and Peterman 1929) ;

(*c*) precipitation with hydrogen sulphide in presence of tartaric acid which retains aluminium hydroxide in solution ;

(*d*) precipitation with nitroso-β-naphthol after removal of phosphates (Ilinsky and von Knorre 1885) ;

(*e*) precipitation of iron with 8-hydroxyquinoline (' oxine ') in certain concentrations of di-carbonic acids with which the aluminium compound of hydroxyquinoline forms soluble complexes (Lehmann 1931).

(iii) Reduction of iron to the ferrous state and precipitation of aluminium as phosphate in dilute acetic acid in which ferrous phosphate is soluble. The reduction is carried out by heating with sodium thiosulphate or bisulphite (Carnot 1891, Schmidt and Hoaglund 1912, Levy 1931). A somewhat similar method is that adopted in the A.O.A.C. handbook (1945) for aluminium in baking powder, in which aluminium hydroxide, with or without phosphate, is precipitated with phenylhydrazine sulphite, ferrous iron remaining in solution.

(iv) Co-precipitation of aluminium and iron as phosphates or hydroxides and determination of iron in the precipitate, aluminium being obtained by difference (Massatsch and Steudel 1930). This method is not exact for small amounts of aluminium since the difference figure has to carry all the errors of experiment. It is however adopted as an official method by the Association of Official Agricultural Chemists (1945) for aluminium in plants.

DETERMINATION OF ALUMINIUM AFTER SEPARATION OF IRON. After separation from iron, aluminium may be determined

(i) by precipitation as hydroxide after removal of phosphoric acid as phosphomolybdate (Berthelot and André 1895) ;

(ii) colorimetrically with alizarin (Atack 1915, Underhill and Peterman 1929) or with aurin tricarboxylic acid (Myers, Mull and Morrison 1928, Lampitt and Sylvester 1932) ;

(iii) precipitation as phosphate (Schmidt and Hoaglund 1912);

(iv) precipitation with 8-hydroxyquinoline ('oxine') either in ammoniacal tartrate or in acetic acid solution (Berg 1927).

Colorimetric methods have been used by a number of workers and depend on the readiness with which aluminium forms coloured lakes with dyes of the alizarin and triphenylmethane series. Levy (1931) criticises Atack's alizarin method as being very liable to error with small amounts of aluminium. Iron must be completely removed as it also gives a red colour with alizarin. Manganese, which is present in most plants, gives a similar colour. Hammett and Sottery (1925) introduced aurin tricarboxylic acid, the colour of which is changed from pale orange to intense red by aluminium. This method was modified and standardised by Lampitt and Sylvester whose paper in the *Analyst* should be consulted for details (cf. also A.O.A.C. methods of analysis 1945). The method is capable of determining amounts of aluminium down to 2 or 3 p.p.m. in 10 g. of food or possibly even less. Lampitt and Sylvester also used a spectroscopic method which, although not nearly so accurate as the colorimetric, provided specific confirmation that the aurin colour was really due to aluminium and that the amount found by the colorimetric method was of the right order of magnitude.

DETERMINATION WITH 8-HYDROXYQUINOLINE. This reagent, introduced by Berg in 1927, has been used for the determination of a number of metals, by varying the conditions under which the precipitation is effected. For the determination of aluminium, this is first separated in the form of the mixed phosphate with iron, the iron is then removed as basic acetate, and aluminium precipitated in an acetic acid solution containing ammonium tartrate.

About 25 to 100 g. of the sample, according to the aluminium content, are ashed with a few drops of sulphuric acid in a silica dish at a low heat, preferably in a muffle furnace. The sulphated ash is extracted with hydrochloric acid. If there is an insoluble residue, and it is desired to include any insoluble aluminium in the total, the residue must be fused with alkali in a platinum dish and the melt acidified and added to the main portion. The solution of the ash is evaporated to dryness in a silica dish and the residue heated to dehydrate any silica present. It is then again extracted with hydrochloric acid and any iron which it may contain oxidised with nitric acid. If there is not enough iron to give the solution a yellow tinge, one drop of a 5 per cent ferric chloride solution is added. It is also usually necessary to add about 0·1 g. of ammonium phosphate.

The solution, which should measure about 20 ml., is treated with a few drops of thymol blue solution and ammonia added until a slight permanent precipitate or turbidity is produced. It should still be slightly acid and red to orange in colour. 3 ml. of glacial acetic acid are added and the solution heated to boiling-point. The precipitation of the mixed phosphates of iron and aluminium is then completed by adding 5 ml. of 50 per cent ammonium acetate solution to the hot liquid gradually with stirring. The solution is now yellow, i.e. still faintly acid. It is filtered, or preferably centrifuged, the precipitate washed with hot water and dissolved in the least possible quantity of warm dilute hydrochloric acid, and the solution diluted to about 15 ml. Iron is now precipitated as basic acetate with 1·25 ml. of glacial acetic acid and 6·5 ml. of 25 per cent sodium hydroxide solution (free from aluminium). After standing for about half an hour with occasional shaking the liquid is filtered or preferably centrifuged and the filtrate taken for determination of aluminium. If there is much basic iron acetate it should be redissolved and re-precipitated and the filtrate added to the main portion.

The alkaline filtrate containing aluminium is diluted to about 30 ml. and made just acid to bromthymol blue with acetic acid. Any slight precipitate of aluminium phosphate which may form at this stage is redissolved by adding powdered tartaric acid. 5 ml. of a 50 per cent ammonium acetate solution are then added. A solution of 8-hydroxyquinoline is prepared by triturating 2·5 g. with 5 ml. of glacial acetic acid, diluting to 100 ml. with water at 60° and filtering. Each millilitre of this reagent will precipitate about 1·4 mg. of aluminium. If more than 2 ml. are required to precipitate the whole of the aluminium it is advisable to dilute the solution to 50 or 60 ml. After gradual addition of a slight excess of the hydroxyquinoline reagent the solution is neutralised with dilute ammonia and finally made just acid with dilute acetic acid. The beaker is covered with a watch-glass, heated to about 70° for 15 minutes and allowed to stand at room temperature overnight.

The greenish-yellow precipitate is filtered off, washed with warm water and dissolved in about 10 ml. of hot hydrochloric acid (4 vols. of acid to 1 of water). The solution is cooled and titrated with 0·1N bromide-bromate solution using indigo-carmine as indicator. An excess of roughly 0·5 to 1 ml. of the bromine solution is added and the solution diluted and titrated with thiosulphate after adding potassium iodide and starch. 1 ml. of 0·1N bromine solution is equivalent to 0·225 mg. of aluminium. Great care must be taken that all reagents are free from aluminium derived from glass. Sodium hydroxide solution should be kept in waxed bottles. With the quantities of reagents specified above, blank tests usually show from 0·13 to 0·25 mg. of aluminium.

The only common metals that may, if present, be precipitated with iron and aluminium as phosphates are titanium, bismuth, tin and lead. Titanium and bismuth are removed with iron in the basic acetate separation. Tin and lead may pass into solution with aluminium in dilute sodium hydroxide, but tin is not precipitated by 8-hydroxyquinoline in weakly acid solution. Lead gives a purple-brown precipitate which is more soluble than the aluminium compound, and amounts of lead up to nearly 1 mg. may be neglected. If more lead is present it must be separated with hydrogen sulphide before precipitating the mixed phosphates.

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18

[SILVER]

TRACES of silver can be shown by the spectroscope to be present in human organs, blood, milk, etc. Kent (1942), using a spectrographic method (cf. p. 97), found 0·3 p.p.m. in wheat flour and 0·9 p.p.m. in bran, figures which are considerably higher than had previously been suspected. Ramage (1930) states that silver occurs regularly in mushrooms in amounts up to several hundred parts per million. It is one of the metals which, although occurring sparingly in soil, becomes more concentrated in the humus from plant remains. Goldschmidt (1937) cites the ash from beech humus in one locality as containing 5 p.p.m. of silver.

Silver may gain access to food from various sources such as silver-plated plant and vessels, silver-lead solders, silver foil used in decorating cakes and confectionery, and more recently the so-called 'Katadyn' process for the partial sterilisation of liquids. The daily intake of silver in foods of all kinds has been given as about 0·06 to 0·08 mg. (Kehoe and others 1940).

THE KATADYN PROCESS. Heavy metals, particularly copper and silver, when present in very small amounts in solution, have a pronounced bactericidal action. This is often referred to as 'oligo-dynamic' action, a term introduced by the botanist Naegeli in 1893. The bactericidal property of silver was applied by Krause (1928) to the disinfection of water and afterwards extended to the treatment of vinegar, mineral waters, fruit juices, wines, etc. Krause's 'Katadyn' silver is a finely divided spongy form of metallic silver with a surface of 1·6 square metres per gramme, obtained by the reduction of silver salts under certain conditions. Water in contact with silver in this form quickly takes up about 0·05 p.p.m., but if the water is circulated between silver electrodes through which a weak electric current is passed, a very much larger quantity of silver can be brought into solution in the form of a metallic hydrosol.

One form of Katadyn apparatus consists of a glass vessel in which two cylindrical silver electrodes are secured with vulcanised rubber fittings. A weak current (30 to 100 milliamperes) is passed through the cell, the voltage being kept well below 1·6 to avoid gaseous decomposition of water. The liquid to be treated is introduced at the bottom of the cell and passes up between the electrodes and out at the top of the cell. The amount of silver dissolved can be regulated by varying the amperage of the circuit and the speed of flow of the liquid. In practice it is found to be only about half that which would be expected from Faraday's law of electrolysis. For each milliampere-hour of current about 2 mg. of silver are dissolved, the actual amount varying with the particular form of apparatus and the nature of the liquid (Brandes 1934). In tests with vinegar Kreipe (1933) found the following figures,

Time in seconds for one litre flow	*Current in milliamperes*	*Tension in volts*	*Silver mg. in one litre of vinegar*
8	31	0·5	0·165
8·4	55	0·5	0·308
14	110	1·1	1·027

It would seem that there is no special virtue in silver produced by the Katadyn process which is not shared by ionic silver from other sources. The bactericidal activities of metallic silver, colloidal silver, silver oxide, silver nitrate, etc., depend upon the amount of ionised silver that they contribute to the solution (Just and Szniolis 1935, Gibbard 1937, Tracy, 1941).

Tests carried out by Tracy with different strains of the coli-aerogenes-proteus group, the typhoid-paratyphoid-dysentery group and *Staphylococcus aureus* indicated that difference in species among non-sporing bacteria has little influence on resistance to the germicidal action of silver, and that this is dependent rather upon cell population and content of organic matter. Other authorities have found a good deal of variation among different organisms, and even among different strains of the same organism, in their susceptibility to silver (Gutschmidt 1935). Gram-negative bacteria (e.g. *B. coli*) are stated to be the most easily destroyed, Gram-positive (e.g. *Staph. aureus*) being resistant. Spore-forming bacteria and most of the air-borne organisms are highly resistant. Sporing organisms are sometimes even activated by silver and may multiply at a greater rate in water containing traces of silver than in untreated water. Mould spores are resistant to silver, but yeasts are fairly easily killed unless they have formed spores. Wild yeasts, being prone to spore formation, are less easily killed than cultivated yeast. Some organisms, such as *Bacterium xylinum* in vinegar, may gradually become acclimatised to increasing doses of silver. High temperature and acidity favour the bactericidal action.

Water, if free from turbidity or from dissolved colloidal matter, and if it contains no organisms resistant to silver, can be sterilised with 0·05 p.p.m. in from 10 minutes to 2 hours, depending on the extent of pollution (Brandes 1934, Callister 1935), but usually a rather larger quantity is used to ensure effective treatment in a short time. For complete sterilisation of water in practice Kamphausen (1939) found that four hours' treatment with 0·5 to 2 p.p.m. of ionic silver was necessary. Tracy (1941) gives 0·1 to 0·5 p.p.m. as sufficient to kill *B. coli* in 1 hour when present in water to the extent of 100 to 100,000 cells per millilitre. Swimming baths have been adequately disinfected with 0·15 p.p.m. of silver daily in the make-up water. Water containing 0·4 to 0·6 p.p.m. of ionic silver is stated to be highly germicidal and has been recommended for use in washing and dressing meat and for cleaning brewery and dairy utensils (Brandes 1934).

In liquids containing colloidal matter or proteins, such as vinegar, wine, fruit juices and beer, the amount of silver required for effective treatment is much greater than for water, since much of it is absorbed by the colloid particles. For instance, Siebenmann (1939) found that silver foil, although possessing bactericidal properties in water, did not inhibit the growth of 15 out of 16 pathogenic microorganisms in broth culture. Oettel (1934) found that it required 10 p.p.m. of silver to kill *B. coli* in a meat infusion ; amounts under 2 p.p.m. actually stimulated growth. According to Noetzel (1939) 0·5 p.p.m. is effective for stabilising fruit juices and vinegar ; Mehlitz (1936), on the other hand, speaks of much larger doses, 5 p.p.m. for clear grape juice, and says that with 7·5 p.p.m. the juice may develop a metallic taste which disappears upon storage for several months. Partially clarified juices may require as much as 20 p.p.m. of silver, most of which, however, separates out in combination with organic matter on storage. Jendrassik and Papp (1935) say that vinegar requires 4 p.p.m. of silver effectively to retard bacterial action, but that most of it separates out in the form of a flocculent precipitate with organic matter. It would seem that an initial high dosage of silver is usually given to these products to effect sterilisation, and that on storage most of it is gradually precipitated, leaving eventually about 0·1 to 0·2 p.p.m., or at most 0·5 p.p.m., in solution.

Precipitation of silver by colloidal protein matter may be more or less delayed according to the nature of the liquid, and moreover such products are not always clarified before sale for consumption. Statements as to the amount of silver required for the treatment of any particular product should be critically examined to see whether they refer to the initial dosage or to residual silver in the clarified product.

In Matzka's patents (1927, 1937) it is claimed that potable liquids such as fruit juices, beer and milk, also liquid eggs and the like and, in fact, any liquids containing colloidal matter, can be sterilised by passing them at a temperature below 80° through a system of concentric tubes of two different metals, with special arrangements for ensuring a turbulent flow and intimate contact of the liquid with the metallic surfaces. A weak electric current, at a voltage not more than 1 to 1·5, flows between the tubes through the liquid. Matzka seems to have used iron, copper, gold and silver anodes at various times but in his latest patent preference is given to silver, the cathode being of aluminium or nickel. The sterilisation of the liquid is stated to be due to silver ions. It is noteworthy that he claims sterilisation of highly colloidal liquids, which would seem to imply a very intense treatment and the solution of relatively large amounts of silver. He gives no indication of the quantity of silver which may remain in the liquid. The apparatus in its various forms appears to be widely used on the Continent for the treatment of fruit juices. In this country the Matzka and Katadyn processes would probably be held to be an infringement of the Preservatives Regulations, if any appreciable amount of silver remains in the food as sold. If they were permitted it would seem that some degree of control would be necessary. In Germany it has apparently been ruled that 1 p.p.m. is to be considered a practical limit for silver, in conjunction with a declaration that the article in question has been treated by the Katadyn process.

According to Beavens and others (1937) metallic silver ‘ ages ’ brandy, although it does not cause any significant change in the acid, ester, aldehyde or higher alcohol content.

The ingestion of silver in quantities of the order of 1 mg. daily could hardly produce any ill effects, and probably much larger amounts could be taken with impunity, but it must be borne in mind that toxic metals are more readily absorbed from liquid than from solid foods, and that little is known of the effects of continued ingestion of small quantities of silver. No doubt its absorption is limited by the solubility of silver chloride in the intestinal fluids. Further knowledge is required of the extent to which silver in these minute amounts may be absorbed from the intestines and its fate after absorption ; also whether, as has been suggested, it may possibly exert any significant inhibitory action on the enzymes of the digestive tract. Many enzymes, e.g. lipase, phosphatase and diastase, are affected by traces of heavy metals.

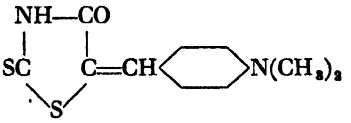
Industrially, silver is held to be a non-toxic metal, but it may be deposited in the tissues from which it cannot be eliminated.

METALLIC SILVER IN PLANT CONSTRUCTION. Pure silver is finding an increasing number of applications in the construction of plant and vessels used in food manufacture. It has distinct advantages over many other metals in that it is not readily corroded by organic acids, even by hot acetic acid ; it does not discolour food products or act as an oxidation catalyst, and its surface is readily kept sterile. Silver, either alone or as a lining or plating, is used for boiling pans, cooling and heating coils, pipes, taps and valves, stirrers, stills and condensers. It is particularly useful for plant used in pickle making.

SILVER FOIL IN CONFECTIONERY. The small sugar beads used by confectioners for decorating cakes, etc., and known to the trade as ‘ nonpareils ’, are sometimes coated with pure silver. Nonpareils of about 4 mm. diameter may show about 0·6 per cent of metallic silver. Another ‘ silver ’ coating often used consists of aluminium containing 10 per cent of copper. The metallic coating can readily be separated by treating the nonpareils with water.

THE DETERMINATION OF SILVER IN FOOD

COLORIMETRIC DETERMINATION. Silver in traces such as occur in food is determined colorimetrically with *p*-dimethylamino-benzalrhodanine



with which it forms a red compound (Feigl 1928). A similar compound is formed by mercury. Strafford (1933) finds that if a solution containing traces of silver and mercury is made strongly alkaline with ammonia and then distinctly acid with acetic acid, and vigorously shaken with a saturated solution of the reagent in amyl alcohol, the red silver complex alone is formed and collects above the interface of the two solvents. Mercury does not interfere unless the amount present is greatly in excess of the silver, e.g. 10,000 parts of mercury to 1 part of silver. The test will detect as little as 2 μg. of silver in 10 ml.

For a quantitative determination the aqueous layer is removed, the amyl alcohol washed with water, and the red complex dispersed to form a colloidal suspension by the addition of ethyl alcohol and glycol. The solution is diluted to a definite volume with alcohol and compared with standards produced in a similar manner.

Jendrassik and Papp (1935) proceed as follows. Organic matter is oxidised in the usual way with nitric acid and a small quantity of sulphuric acid, and the residual liquid diluted with about 25 ml. of water, made slightly alkaline with ammonia and filtered. Any excess of ammonia is driven off by heating on a steam bath and the solution neutralised exactly to litmus paper with nitric acid. It is cooled and made up to 100 ml. 10 ml. of this solution are measured into a test tube or Nessler tube and made up to 50 ml. with water, and 0·2 ml. of a 3 per cent solution of *p*-dimethylamino-benzalrhodanine in acetone added. This quantity is sufficient to react with about 20 μg. of silver. An excess of the reagent must be avoided, or its yellow colour will interfere with the determination.

In another tube is measured an amount of standard silver solution corresponding to 10 μg. of silver. This is made up to 50 ml. with water, 0·2 ml. of the reagent added and the colours compared. If the colour of the test is equal to or stronger than that of the standard a further 0·1 ml. of reagent solution is added to both tubes. If the colour of the test is still the stronger of the two, another tube is prepared with 20 μg. of silver and 0·3 ml. of reagent solution. If the colours are equal or if the test solution is still the stronger, a further 0·1 ml. of reagent solution is added to both. In this way the test solution is progressively compared with standard solutions, increasing in strength by successive stages of 10 μg. of silver and 0·1 ml. of reagent solution until the colours match, both tubes containing the same amount of reagent without an excess being present. If the test solution at the first comparison shows less silver than the standard the operation is repeated with 50 ml. of the original solution undiluted.

A somewhat similar technique with slight variations is recommended by Noetzel (1939).

COLORIMETRIC DETERMINATION AS COLLOIDAL SILVER. In this method, due to Jelley (1932), an ammoniacal silver solution is reduced by sodium hyposulphite (' hydrosulphite ') in the presence of gelatin to form a clear yellow solution of colloidal silver. The method is stated to be rapid and accurate for very dilute solutions of silver, but copper, cobalt, nickel and cadmium interfere and must be separated if present.

DETERMINATION WITH DIPHENYL-THIOCARBAZONE (DITHIZONE). Silver shares with mercury and copper the property of being extracted from acid solution by shaking with a solution of dithizone in chloroform or carbon tetrachloride. It may be determined in presence of mercury and copper by first extracting the slightly acid solution (free from hydrochloric acid) with successive quantities of a standard solution of dithizone in chloroform until the reddish-orange colour of the metal-dithizone compounds gives place to the green of unchanged dithizone, showing that the extraction is complete. To another portion of the original solution enough sodium chloride is added to combine with the silver, and the dithizone extraction is repeated until a similar end-point is reached. The difference between the quantities of dithizone solution used in the two extractions is a measure of the amount of silver present. The dithizone solution is standardised against a very dilute silver solution.

DETECTION OF SILVER WITH 2-THIO-5-KETO-4-CARBETHOXY-1 : 3-DIHYDROPYRIMIDINE. This reagent was introduced by Sheppard and Brigham (1936). It gives precipitates of different colours with various metallic ions in neutral solution but in acid solution only with silver ions. When a solution of the reagent in acetone is added to a very dilute nitric acid solution of silver a purple colour is produced. The preparation of the reagent and details of its use are given in the *Analyst*.

POTENTIOMETRIC DETERMINATION OF SILVER. Hosenfeld and Engelhardt (1938) give a brief outline of a potentiometric method of determining small quantities of silver in solution but give no analytical figures, nor do they say whether the method can be applied direct to fruit juices, etc., or whether organic matter must first be destroyed. The method is similar in principle to other well-known potentiometric titrations. The liquid containing silver is placed in a vessel in contact with an electrode of silver wire coated with silver sulphide. This vessel is connected through a liquid junction with a calomel electrode, and the electromotive force of the cell thus formed is balanced on a Wheatstone bridge against that of a standard cell, using a galvanometer as zero indicator. The silver electrode is positive and the calomel electrode negative. The silver solution is now titrated with very dilute standard potassium iodide solution (1 ml. equivalent to 100 μg. of silver). Silver ions are thus removed from solution as silver iodide, and the electromotive force of the cell gradually falls. The end-point of the titration is the point at which the e.m.f. of the cell suddenly falls to a much lower value owing to the virtual disappearance of silver ions from the solution.

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[CADMIUM]

CADMIUM is one of the more toxic metals. It occasionally finds its way into food from cadmium-plated vessels and appliances and has been responsible for several cases of poisoning. Traces of the order of 1 p.p.m. or less probably occur naturally in many plant and animal tissues (Maliuga 1941). Samples of beef kidney have shown 0·66 p.p.m. and kidneys of other animals smaller amounts (Klein and Wichmann 1945).

CADMIUM PLATING. Cadmium plating was introduced about twenty years ago. It is an excellent protection for copper and brass, taking a high polish and being permanent in air. On iron and steel it is claimed to give better protection than zinc against rust, although there seems to be some difference of opinion whether a bright electrolytic zinc coating is not superior to cadmium for some purposes.

Cadmium stands between iron and zinc in the electromotive series, being only slightly anodic to iron with a potential difference of less than 0·1 volt, and it is possible that it may be cathodic to some kinds of iron or steel plate, thus allowing the latter where exposed to become corroded. Cadmium plating is specially resistant to attack by alkalies.

The usual thickness of the coating on iron and steel is from 0·0001 to 0·0002 inch (0·0025 to 0·005 mm.), corresponding to about 200 to 400 mg. per square decimetre of surface. This is a heavier coating than that of tin on ordinary tin-plate. To determine the thickness of the deposit on steel, cadmium may be stripped from the plate (*a*) with a solution of iodine in potassium iodide, (*b*) with a 5 per cent solution of ammonium persulphate containing 10 per cent of 0·880 ammonia, which does not attack steel, or (*c*) with hydrochloric acid containing antimony trichloride (Clarke 1933).

In course of time cadmium diffuses to some extent into the iron of the plate forming a layer of a cadmium-iron alloy. Steel plate is also often coated electrolytically with a zinc-cadmium alloy containing about 8 to 10 per cent of cadmium, and with a ternary zinc-tin-cadmium alloy.

Cadmium plating is widely used for washing-machines, electric cooker parts and refrigerator trays. When heated the metal is oxidised to the brown monoxide, and one of the dangers connected with its use in electric cookers is that if the part in question becomes too hot, cadmium oxide may become detached and fall into food.

SYMPTOMS OF CADMIUM POISONING. Cadmium salts cause violent and acute gastritis almost immediately after ingestion, with vomiting and diarrhoea. Their action is similar to that of zinc salts, but much more severe. Recovery, as with zinc poisoning, is fairly rapid, since practically all the cadmium ingested is eliminated by vomiting. In general it may be said that acute poisoning by metallic irritants, such as cadmium, zinc and antimony, is distinguished from bacterial food poisoning by the quickness of onset of the symptoms and absence of an incubation period. On the other hand, the action of preformed bacterial toxins, such as that of *Staphylococcus aureus*, may be just as quick as that of metallic poisons.

Chronic poisoning from continued absorption of small quantities of cadmium compounds occurs among industrial workers, especially through inhalation of dust, but has not so far been observed to result in man from consumption of contaminated food. From experiments on cats carried out by Prodan (1932) it would appear that the liver and kidneys are affected ; cadmium is excreted slowly through the kidneys and through the intestinal wall. There are no definite blood changes but the bones retain a relatively large amount. Wilson and DeEds (1939) found that experimental chronic cadmium poisoning in rats was followed by bleaching of the enamel of the teeth, anaemia and cardiac hypertrophy. Sutton (1939) found that 250 p.p.m. of cadmium in rats' food caused anaemia and complete loss of fertility, and Fitzhugh and Meiller (1941) that 45 p.p.m. for a period of 6 months caused slight toxic symptoms.

ACTION OF FOODS ON CADMIUM. According to Beythien (1936) cadmium is readily attacked by 0·25 to 0·5 per cent acetic acid at room temperature and also by boiling jam. Fortner (1932) records that some Slovakian wine which had been passed through a filter press of copper and brass recently plated with cadmium caused symptoms of poisoning. It is possible that the danger of poisoning is not so great with old plating as with new, since the cadmium coating gradually diffuses into copper and brass, becoming thereby somewhat yellow. Fortner and Lukas (1934) give the following solubilities in water of the cadmium salts of organic acids ; cadmium lactate 10·11 per cent, succinate 0·37 per cent, tartrate 0·1 per cent, citrate 0·23 per cent and oxalate 0·009 per cent. Formenti (1931) finds that appreciable amounts of cadmium are dissolved in a few hours by 1 per cent solutions of tartaric, citric or lactic acids and by preserved fruit or tomatoes. Hazen (1934) records an instance in which a gelatin product allowed to solidify in a tray coated with an alloy containing 56 per cent of cadmium, absorbed enough of the metal to cause violent sickness.

Frant and Kleeman (1941) give details of five outbreaks of cadmium poisoning in the United States due to eating lemonade jellies, ices, etc., containing from 15 to 530 p.p.m. of cadmium derived from refrigerator trays and from cadmium-plated vessels. Over 200 cases of poisoning are reported to have occurred among United States Navy personnel from food which had been in contact with cadmium-plated vessels for periods ranging from 1½ to 14 hours. One batch of lemonade contained 100 p.p.m. It was concluded that 15 p.p.m. in food was capable of producing mild symptoms of poisoning. A similar outbreak of cadmium poisoning, affecting 62 men, from fruit juice contained in a galvanised vessel, is reported by Jenner and Cuningham (1944). Ice cubes made in a cadmium-plated refrigerator and exposed to drippings or scale from the plating have caused poisoning (Schriftner and Mahler 1943).

In this country Taylor and Hamence (1942) have drawn attention to eight cases of poisoning from lemon ices prepared in refrigerator trays. Some ices made experimentally in a similar manner showed 190 and 280 p.p.m. of cadmium. Taylor and Hamence calculated that the ingestion of as little as 3 mg. may cause fairly severe symptoms. As with other toxic metals the effect is probably far more pronounced with liquid than with solid food.

Griebel and Weiss (1931) refer to a case of poisoning from coffee made in a pot plated internally with a zinc-cadmium alloy. The pot had been treated with hydrochloric acid to remove scale, and some of the scale had been left behind and had absorbed cadmium chloride.

Larsson (1936) records serious poisoning in Sweden from cooking food in a cadmium-plated cast-iron roasting pan. Cadmium plating of food utensils is now prohibited in Sweden.

Other sources from which cadmium may possibly gain access to food are soft solders and fruit insecticides. A solder sometimes used contains 25 parts of cadmium, 50 parts of tin and 25 parts of lead, and a standard German solder 10 parts of cadmium, 10 of tin and 80 of lead. These solders show a tendency to oxidise which may be checked by the addition of 1·5 to 3 per cent of zinc. Lench and Pilkington (1943) have investigated the action of various foods on solders containing cadmium and find that the metal is readily dissolved, especially by acid foods.

Cadmium compounds have been recommended as insecticides for fruit and the oxide and hydroxide are stated to compare well with lead arsenate, particularly in their toxicity to larvae (Ginsberg 1934). Cadmium salts of organic acids have also been suggested as disinfectants for seeds (Migrdichian 1935).

The use of compounds of cadmium for colouring food is prohibited under the Preservatives Regulations 1925–7, but there are no regulations in this country prohibiting or limiting its use in the plating of articles which may come into contact with food.

THE DETERMINATION OF CADMIUM IN FOOD

COLORIMETRIC DETERMINATION AS SULPHIDE. Cadmium in foods is usually determined as the yellow sulphide insoluble in hydrochloric acid. The liquid obtained after wet oxidation of organic matter is neutralised, made slightly acid with hydrochloric acid and treated with hydrogen sulphide. The precipitated sulphide is distinguished from those of tin, antimony and arsenic by being insoluble in ammonium sulphide. If copper is also present the mixed sulphides are dissolved in nitric acid and cadmium reprecipitated from the filtrate in presence of an alkaline cyanide. Fairhall and Prodan (1931) find that the yellow colour of cadmium sulphide is much more easily seen in the light of a quartz mercury vapour lamp than by daylight. The colour due to 2 p.p.m. of cadmium sulphide in the liquid under examination is almost invisible by ordinary light, but as little as 0·2 p.p.m. is perceptible under a mercury lamp. For the colorimetric determination of cadmium as sulphide Fairhall and Prodan destroy organic matter by wet oxidation with nitric and sulphuric acids using about 10 ml. of sulphuric acid. The residue is diluted to 75 ml., and an amount of copper salt equivalent to 0·5 mg. of copper is added to entrain the cadmium sulphide precipitate. 2 g. of sodium citrate are added to prevent precipitation of iron, the liquid is neutralised with ammonia, and the acidity is adjusted to pH 3·0 with the aid of thymol blue and bromchlorphenol blue. Hydrogen sulphide is passed through the solution and one drop of 5 per cent aluminium chloride added, which prevents the cadmium sulphide precipitate from becoming colloidal and passing through the filter when it is washed. The liquid is allowed to stand for at least six hours. It is then filtered, the precipitate dissolved in aqua regia and the solution evaporated to dryness. The whole operation of hydrogen sulphide precipitation is repeated twice, the second repetition being varied by omitting the addition of sodium citrate and by adjusting the liquid to pH 2·0 instead of pH 3·0 with potassium hydroxide. The final solution of cadmium chloride is evaporated to dryness and the residue taken up with water to a known volume. An aliquot part is diluted with water in a Nessler cylinder and treated with 5 drops of 10 per cent potassium cyanide solution (to immobilise copper) and 5 ml. of a solution of hydrogen sulphide. The colour, which should appear bright clear yellow under the mercury lamp, is compared with standards. Darkness or turbidity of colour indicate incomplete removal of iron.

Jusa and Langheim (1937) determine cadmium colorimetrically as sulphide in ammoniacal solution with the aid of gelatin as a protective colloid.

According to Krumholz and Kruh (1935) the precipitation of cadmium selenide is a more delicate test than that of the sulphide. The reagent is a 1 per cent solution of sodium selenide in water containing 0·5 per cent of potassium cyanide. Details of sensitivity and interference of other metals are given in the *Analyst*.

DETERMINATION WITH β-NAPHTHOQUINOLINE AND POTASSIUM IODIDE. In this method (Berg and Wurm 1927) cadmium is precipitated as the compound $(C_{13}H_9N)_2H_2(CdI_4)$. 0·01 mg. of cadmium in 1 ml. of N/10 sulphuric acid just gives a precipitate with 2 ml. of 1 per cent β-naphthoquinoline sulphate solution and 2 ml. of N/10 potassium iodide, so that the delicacy of the reaction is about 1 in 500,000. Chlorine ions lower the sensitivity since the precipitate is somewhat soluble in hydrochloric acid.

To a solution containing about 1 mg. of cadmium in 50 ml. of 2N sulphuric acid, are added in succession 50 ml. of a 10 per cent sodium tartrate solution (to prevent precipitation of tin and antimony), a small quantity of a 2·5 per cent solution of β-naphthoquinoline in N/2 sulphuric acid, a few drops of dilute sulphurous acid and sufficient N/5 potassium iodide solution to precipitate the cadmium. The precipitate is filtered off and washed with a 0·25 per cent naphthoquinoline sulphate solution containing one-tenth of its volume of N/5 potassium iodide solution. The cadmium compound is practically insoluble in this solution. The precipitate is sucked as dry as possible and decomposed with 20 ml. of 2N sodium hydroxide or ammonia solution. The filtrate is acidified with sulphuric or hydrochloric acid and the iodine titrated with standard potassium iodate solution by Lang's cyanide method (p. 196). If other metals of the hydrogen sulphide group are present, twice as much sodium tartrate or ammonium oxalate should be used and the wash water should also contain sodium tartrate.

DETERMINATION WITH BRUCINE AND POTASSIUM IODIDE. Thompson's method (1941) is primarily designed for the determination of cadmium in zinc ores but may be adapted to foods and biological material after destruction of organic matter. The solution containing cadmium in dilute sulphuric acid (about 100 ml.) is treated with 30 ml. of 1 per cent brucine sulphate solution followed immediately by 30 ml. of 10 per cent potassium iodide solution. The liquid is stirred and the precipitate allowed to settle for ten minutes. It is filtered off with suction and washed first with brucine and potassium iodide solution and then with a mixture of ethyl alcohol and toluene (1 : 4) until free from excess of iodide. It is then dissolved in hot water and the iodine content of the organic iodide determined in any convenient manner. Thompson prefers the method of titrating with silver nitrate in neutral or slightly acid (acetic) solution with eosin as indicator, the dye being adsorbed on the silver iodide precipitate. At the end-point there is a change of sign on the particles of the precipitate and an abrupt change in the adsorption of the electrically charged ions of the dye. With eosin the colour change is from yellowish-red to red-violet. The silver nitrate solution is of 0·03N strength and is previously standardised against a cadmium solution of known strength.

Nikitina's method (1938) is similar to Thompson's, but he uses potassium bromide instead of iodide and weighs the precipitate after washing with alcohol and ether. The precipitate has the composition $[(CH_3O)_2C_{21}H_{20}O_2N_2]_2CdBr_2,2HBr$ and contains 9·2 per cent of cadmium.

Korenmann (1937) gives the delicacy of the brucine potassium bromide reaction as 0·12 μg. of cadmium in one drop, or about 1 in 400,000.

DETERMINATION WITH THIO-UREA AND REINECKE'S SALT. Reinecke's salt is the ammonium salt of tetrathiocyano-diammono-chromic acid and has the formula $[Cr(NH_3)_2(SCN)_4]NH_4$. When a solution of this salt is added to dilute hydrochloric acid containing a cadmium salt and some thio-urea there is formed a very insoluble pale pink precipitate consisting of rod-shaped crystals which aggregate to twins, stars and rosettes. From dilute solutions the compound crystallises slowly and the crystal aggregates assume characteristic curved shapes. The formula of the compound is $[Cr(NH_3)_2(SCN)_4]_2Cd(CSN_2H_4)_2$ and it contains 12·47 per cent of cadmium (Mahr and Ohle 1937). The precipitate may be dried and weighed or the chromium determined iodimetrically after conversion into chromate. Fuller details are given in the *Analyst.* Lead, mercury and silver give white precipitates both with Reinecke's salt and with thio-urea, and copper a similar precipitate with thio-urea, but zinc gives no precipitate. Choline also gives a precipitate with Reinecke's salt.

Reinecke's salt is readily prepared from ammonium thiocyanate and ammonium dichromate. 3 g. of ammonium thiocyanate are heated to incipient fusion in a small porcelain dish and 1 g. of powdered ammonium dichromate added. The mixture becomes dark greenish-purple and fuses with effervescence. When the reaction is over the contents of the dish are allowed to cool and rubbed up with 10 to 20 ml. of water using a small pestle. The mixture is filtered and the residual Reinecke salt washed on the filter with small quantities of water until the pink colour no longer lightens. It is dried on a porous plate and can be used for the determination of cadmium without further purification.

DETERMINATION WITH DITHIZONE. Cadmium

may be determined with dithizone on lines similar to those given for zinc, but as traces of zinc are nearly always present in foods it is preferable first to separate cadmium as sulphide (Fischer and Leopoldi 1937). Cholak and Hubbard (1944) have given the following method of separating cadmium from other metals by dithizone extraction.

Organic matter is oxidised with sulphuric and nitric acids and the liquid diluted, treated with ammonium citrate and made slightly alkaline with ammonia (pH 8·3, phenol red). Cadmium and other metals are extracted with dithizone or with di-β-naphthylthiocarbazone in chloroform. The metals are then removed from the chloroform extract by shaking it with N/5 solution of hydrochloric acid. This solution is made strongly alkaline with 5 per cent of sodium hydroxide and about 1 per cent of sodium tartrate, and the cadmium separated from zinc and other metals by a second extraction with dithizone in chloroform. The cadmium dithizone compound passes into the chloroform forming an orange solution while the excess of dithizone passes, as the yellow sodium compound, from the chloroform to the alkaline solution. Cadmium is then again extracted from the chloroform solution by shaking it with N/5 hydrochloric acid which must be absolutely zinc-free. The solution is made slightly alkaline with ammonia, and a final extraction with di-β-naphthylthiocarbazone in chloroform gives an orange solution of the cadmium complex which can be compared with standards or examined in a photometer. Di-β-naphthylthiocarbazone is preferred to dithizone for the first and last extractions, since its solubility and that of its metallic complexes in the aqueous phase is smaller than that of dithizone and this facilitates the extraction. For the intermediate operation, however, dithizone gives the sharper separation of cadmium from zinc and other metals. Strafford, Wyatt and Kershaw (1945) give a similar separation of cadmium from zinc by extraction with dithizone from normal sodium hydroxide solution (cf. also Klein and Wichmann 1945).

With very small amounts of cadmium occurring naturally in food, its presence in the final extract may be confirmed by Feigl and Miranda's test (1944), in which cadmium gives a red precipitate with a reagent prepared from *aa'*-dipyridyl, ferrous sulphate and potassium iodide.

Cadmium differs from zinc in giving a precipitate in dilute ammoniacal solution with sodium diethyl-dithiocarbamate.

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[CHROMIUM]

TOXICITY OF CHROMIUM COMPOUNDS. Chromic acid and chromates, in which chromium in a high state of oxidation forms the anion, are toxic. Potassium dichromate is well known as an irritant poison and as the cause of ‘ bichromate disease ’ in industry. It has been administered internally as a drug, but only in doses of 6 to 12 mg. Brard (1935) tried the effect of mixing small amounts of chromates with a dog's food. The dog received 0·01 g. of chromium daily in the form of potassium dichromate for 3 months. It gradually became anaemic and died with severe anaemia and lesions of the kidneys. For rats the limit of tolerance for potassium chromate in food is given as 0·125 per cent. This corresponds to 340 p.p.m. of chromium in the food or about 3·5 mg. of chromium daily (Gross and Heller 1946).

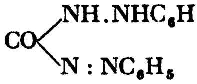
Salts of tervalent chromium, in which the metal is the cation, appear to be relatively non-toxic. Akatsuka and Fairhall (1934) gave as much as 1 g. of chromium daily, in the form of chromium phosphate, to cats for 17 weeks without apparent harm, and Conn, Webster and Johnson (1932) found that chromium is rapidly eliminated by rats and that 100 p.p.m. in the food had no ill effects. On the other hand, Brard (1935), administering 0·5 g. of chromium in the form of trichloride daily to a dog with its food, produced effects similar to those observed with chromates and the dog died in two months. It may be noted that the dose of chromium in this case was fifty times as great as that given in the form of chromate. Possibly the metabolism of chromium trichloride is different from that of the carbonate and phosphate, or Brard's chromium trichloride may have contained some chromyl chloride. Brard considered that the toxic action of chromates is due to the oxidation of haemoglobin to methaemoglobin.

CHROMIUM IN FOOD. Traces of chromium may find their way into food from vessels and plant made from chromium alloys or chromium plate, but as a general rule these materials are very resistant to corrosion. Chromium plating is not sufficiently durable for cooking vessels and the metal is liable to flake off. Titus and others (p. 275) found that when food was cooked or stored in contact with a nickel-chromium-iron alloy containing 18 per cent of chromium the average amount of chromium dissolved was 0·05 mg. from 4 $dm.^2$ of surface. Chromium plating has the advantage over tin plating that fruit stored or heated in chromium-plated vessels is not discoloured as it is by tin-plate (p. 143). In the United States chromium has been recorded as occurring in milk from the use of detergents containing compounds of chromium for cleaning dairy utensils (Verbeck 1940). Tinned vessels are often cleaned with a solution of trisodium phosphate containing sodium chromate (p. 144). Potassium dichromate has been used, chiefly in France, for preserving milk samples for analysis, for which purpose 0·1 per cent of the salt is added to the sample, but there is no record of its having been used in milk destined for consumption.

The reprehensible practice of colouring food with yellow lead chromate still persists in occasional instances, such as the colouring of turmeric root used as an ingredient of curry powder (p. 78). The use of chromium compounds for colouring food is specifically prohibited under the Preservatives Regulations 1925–7.

CHROMIUM IN SOILS AND PLANTS. Van der Merwe and Anderssen (1937) have drawn attention to the high content of chromium in certain igneous soils of South Africa and have suggested that it is the cause of the yellowing of the foliage of citrus trees. The soils in question contain from 0·2 to 0·4 per cent of Cr_2O_3 and the leaves of affected trees 2·5 to 7·5 p.p.m. of chromium. Chromium salts have also been found together with vanadium, selenium and arsenic in a soil in the United States (Byers, p. 477), and it was suggested that the deficient fertility of this soil was due partly to its chromium content. Certain soils on serpentine outcrops in Maryland and Pennsylvania are also stated to owe their lack of fertility to the presence of over 0·1 per cent of chromium and nickel salts. Davidson and Mitchell (1940) give figures for eight Scottish soils ranging from 150 to 350 p.p.m. with two others showing about 1,500 and 3,000 p.p.m. (0·15 and 0·3 per cent). Herrmann and Lederle (1942) give values ranging from 73 to 138 p.p.m. of total chromium for sixteen different soils. Robinson (1914) found traces of chromium up to about 0·025 per cent in many soils, and Slater and others (1937) also found it in most of the soils examined by them.

THE DETERMINATION OF CHROMIUM IN FOOD

Diphenylcarbazide Method. Traces of chromium in food are usually determined colorimetrically with diphenylcarbazide, $CO(NH.NHC_6H_5)_2$, which gives an intense reddish-violet colour with chromates in acid (preferably sulphuric acid) solution (Cazeneuve 1900). The active agent may be diphenylcarbazone  $\mathrm{CO}\left\langle\begin{matrix}\mathrm{NH.NHC_6H_5}\\ \mathrm{N:NC_6H_5}\end{matrix}\right.$ which is readily formed from the carbazide by oxidation, but in view of Evans' work (1939) this seems rather doubtful. The reaction is extremely delicate, it being possible to detect chromium in a dilution of 1 in 100 million (Stover 1928).

Van der Walt and van der Merwe (1938) have applied the reaction to the determination of chromium in plant material. The sample is ashed at a low heat, silica removed from the ash with hydrofluoric acid, the residue fused with potassium bisulphate, the melt dissolved and chromium and iron precipitated with ammonia. Chromium is afterwards oxidised to chromic acid with nitric acid, silver nitrate and potassium persulphate ; and iron and aluminium are removed by precipitation with sodium carbonate. The filtrate is acidified with sulphuric acid, and chromate determined colorimetrically with diphenylcarbazide, using a standard potassium dichromate solution. Full details of the method are given in van der Walt and van der Merwe's paper in the *Analyst*. A simplified modification for use with soil extracts is given by Davidson and Mitchell (1940). Essery (1945) gives a somewhat similar method for traces of chromium in beer worts.

If mercury happens to be present it will give a purple colour or precipitate with diphenylcarbazide if the solution does not contain enough mineral acid (p. 464). In the above method mercury will be removed from solution together with iron and aluminium. Akatsuka and Fairhall (1934) avoid interference from mercury and other metals by first separating chromium as lead chromate. The hydrochloric acid solution of the ash of the sample is evaporated to dryness two or three times with nitric acid. The residue is dissolved in dilute nitric acid, the solution neutralised with potassium hydroxide, hydrogen peroxide added and the liquid heated. The precipitate retains chromium obstinately and must be redissolved in nitric acid and again neutralised and oxidised. A third treatment removes every trace of chromium from the insoluble constituents of the ash. The filtrates are combined and made slightly acid with acetic acid, and the chromate is precipitated with lead acetate. Insoluble lead phosphate is also precipitated at the same time, but does not interfere with the determination. The lead chromate is dissolved in hydrochloric acid, the solution diluted and made up to a definite volume, and chromium determined colorimetrically with diphenylcarbazide. The sensitivity of this method is presumably limited by the solubility of lead chromate (p. 95), but if phosphate is present this may assist in the precipitation of chromate.

It is pointed out by Laug (1934) that when glass vessels are cleaned with chromic acid, traces of the acid adhere obstinately to the surface of the glass and can be removed only by boiling with successive changes of water. The possibility that minute amounts of chromium may thus find their way into reagents in the course of analysis should be borne in mind.

SPECTROGRAPHIC METHOD. Mitchell (1940) and Davidson and Mitchell (1940) have described a spectrographic method for the determination of chromium and other trace elements in soils, depending on the fact that the spectral emission of energy is at its maximum in the region of the cathode of an electric arc. The material to be examined is contained in a cavity bored in the end of a carbon electrode, and the cathode region of the arc is focused on to the slit of the spectrograph. The intensity of the spectral lines is measured by comparison with those of an internal standard, usually iron. For details of this method reference should be made to the original papers (cf. also p. 96).

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21

[BISMUTH]

BISMUTH is not often found in foods, but traces may possibly gain access from various sources. It has been used, for example, to the extent of about 4 per cent as a constituent of low-melting solder in canning ; when added in small quantities (0·4 per cent) to tin-plate and tin-foil it confers increased resistance to corrosion by foods (p. 143) ; it may sometimes be used in veterinary medicines and thus find its way in traces into animal products ; bismuth compounds appear to have been added to wines, either as fining agents or to remove traces of hydrogen sulphide. Records of the use of bismuth for ' doctoring ' wines are difficult to find, but its addition was prohibited by the German Weingesetz of 1907, and Seiler (1924) determines it in wine by precipitation as basic carbonate and weighing as oxide, which indicates that the amount added must have been considerable. It may be present in human tissues and excreta after administration as a drug.

To the food analyst bismuth is of interest chiefly owing to the similarity between its reactions and those of lead, and to the possibility that it may on rare occasions be present and be mistaken for lead.

Bismuth is comparatively non-toxic. About 40 to 50 mg. per kilogram is tolerated by rabbits when injected into the muscles (Leonard 1926), but larger doses may cause damage to kidneys. Long-continued dosage by the mouth with 5 to 10 mg. per kilogram daily also leads eventually to kidney lesions in rabbits (Fishback 1937). Bismuth is slowly absorbed from the intestine and excreted to some extent in the urine, but the greater part is excreted as sulphide in the faeces. After long periods of ingestion it is found in the liver and kidneys and is also excreted in the saliva and in the milk. It is said that most of the bismuth retained in the body is stored in the bones, as with lead, but no bismuth was found by Roche Lynch, Slater and Osler (p. 67) in any of the human bones examined by them.

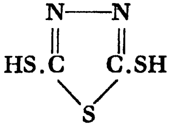
THE DETERMINATION OF BISMUTH IN FOOD

COLORIMETRIC DETERMINATION AS POTASSIUM BISMUTH IODIDE. This is the most usual method of determining traces of bismuth. It has been thoroughly investigated by Haddock (1934). When potassium iodide is added in excess to a solution of a bismuth salt the intensely yellow potassium bismuth iodide, or potassium iodobismuthite, K_2BiI_5, is formed. It can be extracted from acid solution with ethyl acetate giving a red solution. Since ethyl acetate is somewhat soluble in water (9 per cent) it is better to use a mixture of amyl alcohol (3 vols.) and ethyl acetate (1 vol.). If oxidising agents such as cupric or ferric salts are present, iodine may be liberated from the potassium iodide and may interfere with the colorimetric determination. A reducing agent, generally sulphurous acid, must therefore be added to prevent separation of free iodine, but care must be taken not to add too much sulphurous acid since it may itself give a yellow colour with potassium iodide, owing possibly to the formation of iodosulphinic acid, $IHSO_2$. Haddock takes special precautions to avoid a large excess of sulphurous acid and completes the reduction with hypophosphorous acid. Nickolls (1933) prefers to use stannous sulphate as reducing agent in place of sulphurous acid. Portnov and Skvorzov (1928) remove free iodine by shaking with chloroform which does not dissolve iodobismuthous acid, and find that if glycerol is present very little iodine is set free. The colorimetric determination can be made either on the yellow aqueous solution or on the red amyl alcohol-ethyl acetate extract. Organic matter must, of course, be destroyed before the determination, preferably by wet oxidation ; ignition, especially in presence of chlorides, may cause a loss of bismuth.

Several metals and salts interfere with the colorimetric determination. Lead and thallium both give yellow precipitates of the respective iodides, and mercury and antimony, if present in large. amounts, also give yellow compounds which are extracted with amyl alcohol. Chlorides and fluorides bleach the colour, but neither of these will be present after wet oxidation with nitric and sulphuric acids.

According to Haddock the iodobismuthous acid method is suitable for the determination of 0·005 to 0·1 mg. of bismuth. Lead interferes if present in amounts over 0·5 mg. in the solution examined. If considerable amounts of lead or other interfering metals are present, bismuth must first be separated, preferably by one of the methods depending upon extraction with diphenyl-thiocarbazone, or by Strafford's method (p. 188).

DETERMINATION AS DOUBLE IODIDE WITH ORGANIC BASES. Potassium bismuth iodide is a well-known reagent for alkaloids and also for many non-alkaloidal organic bases with which it gives red or orange precipitates of the general formula, BaseHI,BiI_3. Conversely these reactions can be used for the detection and determination of bismuth. Feigl and Neuber (1923) found that cinchonine with potassium iodide will give a red precipitate with 1 part of bismuth in 350,000 of water. Aubry (1922) found that the reaction with quinine was too variable for quantitative application, but Lissievici-Draganescu (1924) used it for determining bismuth in biological material with the aid of gum arabic to keep the precipitate in suspension. Hill (1925) applied the reaction between iodobismuthous acid and antipyrine (phenazone) to the determination of bismuth in urine. Hayes and Chandlee (1939) precipitate bismuth as quinaldine iodobismuthite ; the precipitate is filtered off, decomposed with sodium hydroxide and the iodine determined by titration with standard potassium iodate (cf. p. 196). Berg and Wurm (1927) and Kolthoff and Griffith (1938) precipitate iodobismuthous acid in nitric or sulphuric acid solution with 8-hydroxyquinoline (' oxine '). Oxine itself, without potassium iodide, will also precipitate bismuth in weakly acid acetate or ammoniacal tartrate solution as a crystalline orange-yellow precipitate $Bi(C_9H_6ON)_3,H_2O$ which can be dried at 100° and either weighed or determined by bromide-bromate titration (cf. aluminium, p. 415). 1 ml. of 0·1N bromide-bromate solution is equivalent to 0·001743 g. of bismuth (Berg 1927, Hecht and Reissner 1935, Sazerac and Pouzergues 1932).

REACTIONS WITH ORGANIC SULPHUR COMPOUNDS. Bismuth, in common with other heavy metals, combines readily with a number of organic sulphur compounds, such as ammonium thiocyanate, thio-urea, substituted thio-ureas, dithiocarbamates and thiocarbazones, and mercapto-thiodiazoles. Many of these compounds are intensely yellow or red. Dimercapto-thiodiazole,  gives a characteristic brilliant orange-red precipitate, $Bi(C_2HN_2S_3)_3$, in a dilution of 1 : 28,000, and will detect the presence of 0·001 mg. when used as a drop test (Dubský and Okač 1934). The reagent is readily prepared by dissolving 1 g. of hydrazine sulphate in 15 ml. of water, adding 2 g. of carbon disulphide in 4 ml. of alcohol and then gradually, with shaking, a solution of 1·1 g. of potassium hydroxide in 10 ml. of alcohol. The mixture is heated on a steam bath under a reflux condenser for three hours, filtered from precipitated potassium sulphate and acidified with a few drops of hydrochloric acid. On standing it separates colourless needle crystals of dimercapto-thiodiazole, but it is not necessary to separate the pure substance as the solution is an excellent reagent for bismuth. Other metals of the hydrogen sulphide group give yellow or yellowish-brown precipitates, while those of the ammonium sulphide group do not react. Mercury and lead in particular give bright yellow precipitates. Majumdar (1944) has applied this reaction to the colorimetric determination of bismuth.

DETERMINATION WITH THIO-UREA. Thio-urea gives an intense yellow colour with bismuth in acid solution, and this reaction has been used by Tompsett (1938) for its determination in biological material. It is suitable for amounts of bismuth from 0·005 to 0·8 mg. Organic matter is first destroyed by wet oxidation with sulphuric and perchloric acids and the digest is diluted. At this stage it is necessary to separate bismuth from metallic salts which interfere with the thio-urea reaction. This is done by taking advantage of the solubility in ether of bismuth diethyldithiocarbamate. The solution is treated with sodium citrate to prevent precipitation of iron and phosphates and then made slightly alkaline with ammonia. Sodium diethyldithiocarbamate in water is then added and the bismuth compound extracted with ether. Copper and lead are also extracted but do not interfere with the subsequent determination of bismuth (cf. Strafford's use of diethylammonium diethyl-dithiocarbamate for extraction from *acid* solution, p. 188). The ether is evaporated, the residue oxidised with sulphuric and perchloric acids, an aqueous solution of thiourea added in excess and the yellow colour compared with standards.

Mahr (1933) mentions a number of metals and salts which interfere with the thio-urea reaction, but with the exception of lead and copper they are practically all removed by the above treatment. Lead and copper do not interfere unless present in comparatively large amounts.

DETERMINATION AS THIOCYANATE. For colorimetric determination as thiocyanate, bismuth must of course first be separated from iron, either as sulphide (Heinrichs and Hertrich 1924) or by extracting the red ferric thiocyanate with ether, leaving the yellow bismuth thiocyanate in solution (Hamence 1937). Hamence separates lead and bismuth from other metals by first precipitating with hydrogen sulphide in presence of a ferrous salt at about pH 8·0, dissolving the precipitate in nitric acid and re-precipitating iron as ferric hydroxide with ammonia. The ferric hydroxide takes down lead and bismuth, leaving copper in solution.

It is filtered off and dissolved in dilute nitric acid, ammonium thiocyanate is added and ferric thiocyanate removed by extracting with ether until the ether extract is colourless. Addition of more thiocyanate to the aqueous solution gives the yellow colour of bismuth thiocyanate, and this is compared with standards. If lead is present, and is to be determined, pyridine is added and bismuth extracted in the form of its complex with pyridine thiocyanate with a mixture of ether and amyl alcohol, leaving lead in aqueous solution (cf. p. 91). This determination of bismuth as thiocyanate and its separation from lead as pyridine thiocyanate must be carried out under specified conditions as regards concentration of reagents, etc., for details of which Hamence's papers should be consulted.

DETERMINATION WITH DIPHENYL-THIOCARBAZONE (DITHIZONE). On extraction from neutral or alkaline solutions with dithizone in chloroform or carbon tetrachloride, bismuth forms a red dithizone complex similar in colour to that of lead. In absence of lead or other heavy metals it can be determined colorimetrically. Towards cyanide bismuth shows less affinity than copper and zinc but more than lead. The bismuth-dithizone complex, although unstable in presence of cyanide, is not so unstable as those of copper and zinc. Repeated extraction with dithizone in chloroform removes bismuth from cyanide solutions, but conversely if chloroform containing bismuth-dithizone is repeatedly shaken with cyanide solution bismuth is removed by the cyanide. Thus Haddock (1934) adds relatively large amounts of cyanide to retain copper, silver, zinc, etc., but succeeds in extracting all the bismuth by vigorous shaking with four successive portions of dithizone in chloroform. Tompsett and Anderson (1935), after extracting lead and bismuth with dithizone in carbon tetrachloride, found that all the bismuth could be transferred to the aqueous phase by shaking the carbon tetrachloride four or five times with a 1 per cent potassium cyanide solution. In this way 0·1 mg. of bismuth could be completely separated from 0·01 mg. of lead (p. 87). It appears therefore that there is a state of equilibrium between bismuth cyanide and bismuth-dithizone which can be displaced in either direction by an excess of one or other of the reagents.

If iron or phosphates are present precipitation must be prevented by adding ammonium citrate. This also serves, by de-ionising iron, to prevent oxidation of dithizone.

Haddock (1934) evaporates the chloroform solution, oxidises the residue with sulphuric acid and hydrogen peroxide, and determines bismuth colorimetrically with potassium iodide.

If much lead is present, bismuth can be separated from it, and also from zinc, by bringing the solution to an acidity of pH 2·0 and shaking with a large excess of dithizone in chloroform. The bismuth-dithizone complex is extracted by chloroform at this degree of acidity, leaving lead in aqueous solution (Willoughby and others, p. 87, Clifford and Wichmann 1936). Fischer and Leopoldi give pH 2·8 to 3·0 (bluish tint on Congo red paper) as most suitable for this extraction. Bambach and Burkey (1942) bring the solution to pH 3·4 with hydrochloric acid and potassium hydrogen phthalate.

Hubbard (1939) has applied this principle to the determination of bismuth in biological material. Organic matter is destroyed by wet oxidation, a small amount of copper is added, and bismuth, lead and copper are precipitated as sulphides. The sulphides are dissolved in nitric acid, and the diluted solution brought to pH 9·5 by addition of ammonia and extracted with dithizone in chloroform in presence of sufficient cyanide to retain the copper. The lead and bismuth dithizone compounds are decomposed with dilute nitric acid and the chloroform-dithizone solution rejected. The solution containing lead and bismuth nitrates is brought to pH 2·0 with ammonia and shaken with dithizone in chloroform which extracts bismuth, leaving lead in aqueous solution. Finally the bismuth-dithizone compound is again decomposed with dilute nitric acid, and the bismuth again extracted from slightly alkaline solution with dithizone and determined colorimetrically by methods similar to those given for lead

(p. 93). According to Hubbard 5 μg. of bismuth can be determined in this way.

DETECTION OF BISMUTH AS CHROMATE. This method of detecting bismuth was used by Roche Lynch, Slater and Osler (1934) when examining human bones for lead (p. 67). Lead and bismuth are precipitated together as chromates in dilute acetic acid solution. Sodium hydroxide solution (10 per cent) is added drop by drop to convert the insoluble chromates of the metals into hydroxides. Lead hydroxide is dissolved by the alkali, and any bismuth present remains in suspension. The test will detect 0·02 to 0·05 mg. of bismuth in the presence of 1 mg. of lead.

DETECTION WITH ALKALINE STANNOUS SOLUTION. Bismuth compounds are reduced to metal by an alkaline stannous solution at ordinary temperatures, giving a black precipitate. This reaction has been used for the detection of bismuth in tissues and is stated to be capable of showing 0·01 p.p.m. (Barrenscheen and Frey 1929, Ganassini 1922, Lehman and others 1935).

OTHER METHODS. A number of other methods for determining bismuth have been described, but they are mainly concerned with ores and alloys and are mostly unsuitable for the small amounts in biological materials and food. They include precipitation of bismuth on copper as in the Reinsch test for arsenic (Valiaschko and Virup 1930, cf. also Evans, p. 173), determination as picrate (Etienne 1938), and as phosphate (Little and Cahen 1910, Schoeller and Lambie 1937).

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22

[MERCURY]

WE OWE much of our knowledge of the occurrence and distribution of mercury in foods and biological material, and of its determination in minute traces, to Stock and his co-workers (1926–40). Stock expresses pronounced views as to the high toxicity of mercury vapour in air, e.g. in laboratories. According to him, anything over 0·01 mg. per cubic metre, if breathed for a few hours daily over several weeks, will bring on nervous symptoms, catarrh, fatigue, headache and loss of brain power, and for some individuals a still smaller concentration is toxic. Even amalgam tooth stoppings he regards as dangerous owing to the evolution of traces of mercury vapour. He records that in his own case several weeks' work in an atmosphere containing 0·001 mg. of mercury per cubic metre was sufficient to bring on symptoms of poisoning. He admits that this hyper-sensitiveness to mercury is unusual and probably due to a special allergic condition, but nevertheless maintains that most laboratory workers suffer more or less from mercury poisoning and that 0·01 to 0·02 mg. per cubic metre is always dangerous if breathed daily for several weeks. In this connection it is interesting to note that Bass (1943) refers to three cases of pronounced sensitiveness to metallic mercury in children.

Although it is generally recognised that small quantities of mercury vapour in air may be dangerous, Stock's conclusions as to its extreme toxicity have not been confirmed. Fraser (1934) found from experiments on dogs that 2 mg. of mercury per cubic metre of air, breathed for 8 hours daily over a period of 6 weeks, produced no obvious symptoms. The minimum concentration of mercury vapour required to produce chronic toxic effects was 3 mg. per cubic metre, a figure of quite a different order from that given by Stock. With dogs the proportion of the inhaled mercury retained in the lungs is about 25 per cent only; it does not follow that this is the same for human beings. Göthlin in 1911 was of opinion that the inhalation of 0·4 to 1·0 mg. of mercury vapour daily for several months leads to poisoning, an amount which would be supplied by air containing 0·7 mg. per cubic metre breathed daily for 3 to 5 hours. Presumably, however, a certain proportion of this would be exhaled again. Goodman (1938) gave a figure of 0·25 mg. per cubic metre as the safety limit in air, and Shepherd and others (1941) put it at 0·1 mg. Air saturated with mercury vapour at room temperature contains 15 mg. per cubic metre, but this is found only quite close to a mercury surface, and it is rare to find as much as 0·2 mg. per cubic metre in the air of a laboratory.

DISTRIBUTION OF MERCURY IN FOOD, ETC. According to Stock (1938) mercury is widely distributed in nature in rocks, soils, dusts (especially soot), water, etc. Soil may contain 0·1 to 0·3 p.p.m., soot from 3 to 30 p.p.m. and street dust 0·8 p.p.m. Rain-water contains 0·0002 p.p.m. and sea-water only 0·00003 p.p.m.

Traces of mercury of the order of 0·005 to 0·05 p.p.m. occur in nearly all foods. Vegetables and fruit were found by Stock to contain 0·005 to 0·025 p.p.m., flour 0·025 to 0·035, bread 0·005, potatoes 0·001, vegetable fats 0·06 to 0·115, animal fats 0·07 to 0·28, meat 0·005 to 0·02, kidneys 0·02 to 0·07, eggs 0·002, milk 0·006 to 0·01 and fish 0·025 to 0·18 p.p.m. Stock's estimate of the average amount of mercury normally consumed every day in food is 0·005 mg. Gibbs and others (1941) put it higher, at 0·02 mg. These amounts in food are for the most part extremely small, of the same order as those of iodine, but Stock and his co-workers have devoted so much labour to perfecting the technique for the determination of the smallest traces of mercury that we must accept their statements as to its wide distribution in natural products. Possibly the occurrence of mercury in these minute amounts in food may be due partly to the volatilisation of the small quantity present in coal and its relatively high concentration in coal smoke and soot.

MERCURY IN THE BODY AND ITS EXCRETION. According to Stock (1940) slight storage of mercury takes place in the liver (0·01 to 0·12 p.p.m.), the kidneys (0·04 to 0·10 p.p.m.) and the pituitary (0·04 to 0·13 p.p.m.), the figures given being those for the fresh tissue. Other human organs and tissues show from 0·001 to 0·01 p.p.m. The blood contains from 0·003 to 0·007 mg. per litre. An adult normally excretes from 0·0005 to 0·001 mg. of mercury daily in the urine and up to 0·01 mg. in the faeces. Mercury given by the mouth is excreted mainly in the faeces and only to a slight extent in the urine. It tends to accumulate in the hair, nails and skin.

Metallic mercury and its insoluble compounds are comparatively non-toxic, as shown by the large doses commonly given as drugs, but mercuric chloride and other soluble salts are well known to be highly toxic. Mercury when it gains access to the circulation acts pre-eminently as a kidney poison, causing nephritis. Absorption from the alimentary tract is followed by a rapid rise of the mercury content of the kidneys, from which the metal is only slowly eliminated. Stock considers that if the urine contains more than 0·01 mg. per litre mercury poisoning is probable, and with 0·05 mg. it is certain, but it would seem that the critical concentration is more likely to be of the order of 0·1 to 0·2 mg. per litre.

TREATMENT OF SEEDS WITH MERCURY. Organic compounds of mercury are now widely used for the destruction of mould spores on seeds, particularly cereals, before sowing. The general formula of these fungicides is $R.\mathrm{Hg}X$, where R is a hydrocarbon or substituted hydrocarbon radicle and X an acid anion. They include compounds of mercury with chlorphenol, nitrophenol, cresol, cresol and cyanide, furfuramide, phenyl and ethyl chlorides, phosphates and arsenates, and mercury aminochloride, and are sold under various trade names (cf. *J. Min. of Agriculture*, 1944, **50**, 480). Some of these preparations are solutions containing from 2·5 to 17 per cent of mercury; others are powders containing 1 to 2 per cent of mercury (Kluge, Tschubel and Zitek 1938, Tisdale 1932, Freak 1942). Some of them also contain arsenic and copper. The liquid preparations give a better distribution of mercury on the grain and are more effective as fungicides, but the grain has afterwards to be dried. The treatment of grain with mercury compounds has been criticised in America on the ground that it causes rapid loss of germinating power (Weston 1941), but according to Freak (1942) there is no evidence that dusting with the dry compounds affects germination. It is however possible that the distribution of the powder over the bulk of the grain may be made uneven by subsequent handling, with the result that some portions receive an overdose and germination may be affected (Fitzgibbon 1943).

In grain treated with these preparations according to the usual commercial practice Kluge found from 12 to 25 p.p.m. of mercury. The grain fed to small animals produced slight toxic effects only after long periods of feeding. 0·07 mg. of mercury in the form of mercury-treated grain given daily to rabbits for 3 months did them no harm, and fertility was unaffected. The minimum toxic dose for rabbits was between 2·3 and 5 mg. of mercury daily over a period of 5 months. 6 mg. daily was eventually fatal (Kluge and others 1938). Edwards (1942) records a case of fatal poisoning of a horse through eating treated grain. Wheat grown in districts where mercury treatment of the seed is practised has been found to contain 0·016 to 0·031 p.p.m., a negligible quantity.

The incorporation of yellow mercuric oxide or of mercurous chloride in fertilisers at the rate of 4 lb. per ton reduces scab infestation of potatoes (Cunningham 1936) and also club root in cabbage and allied vegetables (Green and Ashworth 1943).

Mercury vapour, given off from finely divided mercury or from sheets of tin or copper amalgamated on the surface, has been used in India as an insecticide for stored grain (Dole 1943). According to Wright (1944) it is very effective in preventing the breeding of the various insects which infest granaries. Grain stored for several months under these conditions showed no mercury contamination by spectroscopic tests, but it would seem that further investigation on this point would be necessary before the process could be safely adopted for grain intended for human consumption.

TREATMENT OF CONFECTIONERY TRAYS WITH MERCURY. In the annual report of the Chief Medical Officer, Ministry of Health (1920), reference is made to the occasional use of mercury to amalgamate the surface of tin-plate moulds to prevent confectionery from sticking to the metal on cooling. The opinion is expressed that 'if the treatment of the tin is carefully carried out with the minimum quantity of mercury there would appear to be no danger of the sweetmeats being contaminated to an appreciable extent, but it is obvious that there may be considerable risk to the consumer if an excess of mercury is used'. Clearly there is no justification whatever for using mercury for this purpose. It is quite unnecessary, and mineral oil ('slab oil') answers the purpose perfectly.

MERCURY IN WINE. Martini and Berisso (1934) refer to the occasional addition of mercuric chloride to wine to prevent secondary fermentation, which seems a most reprehensible practice.

THE DETERMINATION OF MERCURY IN FOOD

Methods for the determination of mercury in food and biological materials fall for the most part into two classes, colorimetric and micrographic. The latter involves separation of mercury in the metallic state by distillation and measurement of the size of the droplet under the microscope.

DESTRUCTION OF ORGANIC MATTER. In wet oxidation of organic matter by the usual methods mercury may be lost by volatilisation, especially if halogens or halogen ions are present. Strafford and Wyatt (1936) oxidise with sulphuric acid and hydrogen peroxide and pass the volatile products from the oxidation flask through two trap flasks containing, firstly, strong sodium hydroxide solution and, secondly, a hydrogen sulphide solution containing copper sulphide and paper pulp in suspension in order to retain volatilised mercury. Fraser (1934), following a method given by Lomholt and Christiansen (1917), destroys organic matter in urine by adding 30 g. of potassium permanganate and 100 ml. of sulphuric acid to 1 litre of urine and heating for 2 hours at a temperature just below the boiling-point. Any excess of permanganate is then reduced with oxalic acid. If the liquid remains yellow (incomplete oxidation) more permanganate is added and the heating continued until oxidation is complete, excess of permanganate being removed as before. In the resulting solution mercury is precipitated as sulphide. Booth, Schreiber and Zwick (1926) destroy excess of permanganate with hydrogen peroxide.

Winkler (1935) uses a digestion flask fitted with an internal condenser. Stock effects sufficient destruction of organic matter by treatment with chlorine on a steam bath, excess of chlorine being subsequently expelled with a stream of air or carbon dioxide. Kluge and others (1938) destroy organic matter by dry combustion with cupric oxide in a Dennstedt tube, using about 20 g. of the sample, the mercury vapour being trapped in nitric acid.

SEPARATION OF MERCURY AS SULPHIDE. If mercury is to be determined by micro-electrolysis or by micrographic methods it is usually necessary to concentrate it into a small volume and free it from interfering substances. This is best effected by precipitation as sulphide together with cupric sulphide as a carrier. Stock has shown that it is possible to recover as little as 0·00002 mg. of mercury by this method. Fraser adds 5 mg. of copper sulphate for every litre of the solution obtained after oxidation of organic matter, passes hydrogen sulphide through the liquid for several minutes at room temperature, boils it gently for 10 minutes, allows it to stand for 24 hours and separates the precipitate on a centrifuge. Strafford and Wyatt (1936) recommend the addition of paper pulp and filtration through a paper pulp filter. The precipitate of mixed sulphides may be dissolved in aqua regia or in a mixture of sulphuric and nitric acids, sulphur being removed with carbon disulphide. Alternatively it may be dissolved by suspending it in water and passing in chlorine gas, the solution being afterwards aerated for about an hour to remove excess of chlorine. Booth and others (1926) make use of manganese hydroxide as a carrier, adding a manganese salt and precipitating with hydrogen sulphide in alkaline solution.

SEPARATION BY DEPOSITION ON COPPER OR IRON. Mercury may be separated qualitatively from biological fluids or from liquid foods without previous destruction of organic matter by a method similar to the Reinsch test for arsenic (Gettler 1937). The sample is strongly acidified with concentrated hydrochloric acid and the mixture heated on a boiling water bath for half an hour with a small piece of bright copper foil or a copper wire spiral. A dark deposit forms on the copper if arsenic, antimony, bismuth, selenium or mercury are present. Mercury is separated from the other metals by heating the copper to about 300°, at which temperature mercury is volatilised and may be condensed in a capillary tube. Stock separates mercury by allowing an acid solution containing mercury and copper to remain in contact with metallic copper at room temperature for two days.

Evans and Clarke (1926) find that separation on copper is erratic in presence of hydrochloric acid owing to deposition of cuprous chloride on the surface of the copper. They recommend removal of hydrochloric acid by evaporation on a steam bath in presence of sulphuric acid. Mercury is not lost during evaporation. The deposition of mercury on copper differs from that of arsenic, antimony and bismuth in that hydrochloric acid inhibits the mercury reaction but is essential for that of the other metals, while nitric acid favours deposition of mercury but inhibits that of the other metals (Evans).

Bodnár and Szép (1929) and Fraser (1934) separate mercury (in amounts between 0·00004 and 0·01 mg.), in presence of a copper salt and 10 per cent of potassium chloride, by deposition on pure iron wire, and afterwards obtain a globule of metallic mercury from the iron wire by distillation. Details of Fraser's method are given below (p. 461).

ELECTROLYTIC SEPARATION OF MERCURY. Micro-electrolysis is a feature of several methods for detecting or determining traces of mercury. It is usually preceded by precipitation as sulphide and concentration into a very small volume of solution. Booth and others (1926) use as cathode the tip of a pointed copper wire inserted into one drop of the solution. The anode is a thin platinum wire. Electrolysis is carried out with a current of 1·5 to 2·0 milliamperes. If the solution contains 0·5 p.p.m. of mercury (0·025 μg. in one drop) the copper becomes coated with a silver-white amalgam. The mercury deposited on the copper wire is determined by measuring the size of the droplet after distillation into a capillary tube (see below). Stock and Heller (1926), for larger quantities of mercury, use a weighed gold wire cathode. When organic matter has been destroyed by chlorination, ammonium oxalate and oxalic acid added to the electrolyte will prevent interference by chlorinated compounds. Fraser (1934) also used a weighed gold cathode and electrolysed for 18 hours in a small electrolysis tube at a potential difference of 1·3 to 1·4 volts, which is not sufficiently high for the deposition of copper. The microchemical balance used had a sensitivity of 0·02 mg., and Fraser considered that the method was not suitable for quantities of mercury less than 0·2 mg. Young and Taylor (1929) electrolyse the liquid remaining after acid oxidation of organic matter, with two platinum electrodes, the cathode being about 1 cm.^{2} in size. A current of 0·5 ampere is used and the electrolysis allowed to run for 12 hours. The mercury is dissolved from the platinum electrode with fuming nitric acid and determined by titration with 0·05 or 0·01N potassium thiocyanate solution, using ferric ammonium alum as indicator.

Cox (1925) gives a method for detecting traces of mercury in artificial vinegar, in which 200 ml. of the sample are treated with 10 ml. of nitric acid and electrolysed direct, using a platinum anode and a gold-foil cathode with an area of 1 cm.[2] A current of 3 amperes is passed through the liquid for 1 hour. If much mercury is present it shows as a silver coating on the cathode. The gold-foil cathode is folded up and placed in a glass tube drawn out and sealed at one end, into which is placed a fragment of iodine. The open end of the tube is closed with a cork and the tube gently heated from the wide end towards the capillary. Mercury sublimes from the gold-foil and diffuses into the capillary tube, where it meets the iodine vapour and gives a red ring of mercuric iodide on the glass. The limit of the test is stated to be about 0·02 mg.

MICROMETRIC DETERMINATION. In this method mercury is distilled, the vapour condensed in a capillary tube and the size of the globule measured under a microscope. The preliminary stages of the determination are (i) wet or dry oxidation of organic matter, (ii) precipitation as sulphide together with copper sulphide, and (iii) separation on copper or iron wire or by electrolysis on a copper or gold cathode. By using capillary tubes for the distillation and condensation of mercury, it is claimed that as little as 0·00005 mg. can be collected as a coherent droplet and measured under a microscope (Stock and Heller 1926, Bodnár and Szép 1929), but to do this requires a great deal of experience. Fraser's (1934) modification of Bodnár and Szép's method is as follows.

The precipitated sulphides of mercury and copper, suspended in 10 ml. of water, are dissolved with chlorine gas, excess of chlorine being removed by aeration. The liquid is weighed and copper sulphate and potassium chloride added to the extent of 0·05 per cent and 10 per cent respectively. About 100 mg. of the liquid are weighed into a small glass tube 6 cm. long and 1·5 mm. internal diameter sealed at one end. An iron wire 6·5 cm. long and about 0·3 mm. in diameter, previously cleaned with sandpaper, is placed in the tube which is slightly warmed. After a short time the wire is removed and a second similar piece of wire inserted together with 5 to 10 mg. of a 0·5 per cent copper sulphate solution.

The two wires are washed with water, alcohol and ether and the mercury distilled in a capillary tube. This tube is 19 cm. long ; the end portions are 9 cm. and 6 cm. long with an internal diameter of about 1 mm. and the centre portion is 4 cm. long and 0·2 mm. internal diameter. The tube must be thoroughly cleaned with chromic acid, water, alcohol and ether before drawing it out, and if not used immediately must be sealed to prevent access of dust. The two wires are placed in the 6 cm. part of the tube, the open end of which is now sealed off. The tube is gradually heated from the sealed end with a Bunsen burner until it fuses. The condensed mercury is carefully driven with a small flame to the middle of the capillary which is then sealed off on the side next to the closed end. Absolute alcohol is introduced into the open end to a depth of 2 to 3 cm. above the capillary. A right-angled bend is now made about 3 cm. from the open end to serve as a support in the centrifuge. The tube is inserted into a bored rubber stopper in a centrifuge cup and centrifuged for a short time. If the mercury droplets do not unite in the capillary tube they may sometimes be induced to do so by heating in a water bath. The droplet is then moved towards the open end by gently tapping the tube, the sealed end is broken off, the droplet blown on to a microscope slide and its diameter measured. The best results are obtained if the droplet measures between 0·025 and 0·1 mm. If d is the diameter in millimetres, the weight of mercury in milligrams is given by the formula, $(13{\cdot}55 \times \pi \times d^3)/6$. A droplet of 0·025 mm. diameter weighs 0·0001 mg. According to Fraser this is the practical limit of the method owing to the difficulty of getting the distilled droplets to unite. It corresponds to 0·01 mg. of mercury in the 10 ml. of solution containing the mixed sulphides. For amounts larger than 0·5 mg. he recommends the electrolytic method with a weighed cathode (p. 460), but of course by taking a suitable aliquot part the method may be adjusted to any quantity. Qualitatively Fraser claims that the method will detect an extraordinarily minute quantity of mercury.

Booth, Schreiber and Zwick (1926), using a method in which mercury is precipitated as sulphide in alkaline solution together with manganese hydroxide, subject the dried precipitate to combustion with magnesite and lead chromate in a hard glass tube, the mercury vapour being condensed in a standard capillary tube and the length of the thread measured. The method is suited to larger quantities of mercury, 0·25 to 4 mg.

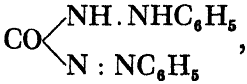
DETERMINATION WITH DIPHENYL-THIOCARBAZONE (DITHIZONE). Mercury, copper and silver, and to some extent bismuth, are the only common metals extracted from an acid solution by dithizone in chloroform. Silver is not extracted if chlorides are present. Mercury is extracted more readily than copper, so that if a solution containing both metals is shaken with this reagent the chloroform first shows the brownish-orange colour of the mercury-dithizone compound and on further extraction the reddish-brown colour of the copper compound. Winkler (1935) has based on this a titrimetric determination of small amounts of mercury in organic materials. Organic matter is first destroyed with nitric acid, potassium permanganate and hydrogen peroxide, using an internal condenser in the neck of the digestion flask to prevent loss of mercury by volatilisation. Excess of nitrous acid is removed by adding hydroxylamine, and the acid liquid is shaken with successive portions of a solution of dithizone in chloroform. If copper is absent the complete extraction of mercury will be shown by a change of colour of the extract from the brownish-orange of the mercury compound to the green of uncombined dithizone. If a small quantity of copper is present the colour of the extract will change from yellow to reddish-brown instead of green.

The combined chloroform extracts are then shaken with a solution of permanganate acidified with sulphuric acid. This oxidises the dithizone, and mercury and copper pass into the aqueous portion, leaving the chloroform only slightly yellow (due to products of oxidation of dithizone). The excess of permanganate is now destroyed by careful addition of hydrogen peroxide and the acid aqueous solution titrated with a standard solution of dithizone in carbon tetrachloride, using a standard mercuric chloride solution for comparison.

1 ml. of the standard dithizone solution contains 0·00125 mg. of dithizone, and is approximately equivalent to 0·005 mg. of mercury. The end-point of the titration is marked by a change of colour from brownish-orange to green, or to red if a trace of copper is present. A detailed description of this method will be found in the *A.O.A.C. Methods of Analysis*, 1945, pp. 470–73 (cf. also Milton and Hoskins 1947).

If much copper is present it may interfere with the titration of mercury. It can be removed by shaking the combined dithizone extracts obtained in the first operation with an acid solution of potassium iodide containing a small amount of arsenious acid to prevent the separation of free iodine. This treatment leaves copper in the chloroform combined with dithizone but transfers mercury to the aqueous phase. Mercury in acid solution cannot be extracted or titrated with dithizone when iodides are present, but this can be done in ammoniacal solution. If therefore the aqueous solution is now made alkaline with ammonia, mercury can be extracted and titrated with standard dithizone solution.

Laug and Nelson (1942) give a similar method in which potassium bromide is used in place of iodide, forming a complex with mercury which is afterwards decomposed by adjusting the reaction of the liquid to pH 6·0, and the mercury extracted with dithizone.

COLOUR REACTION WITH DIPHENYLCARBAZONE. Diphenylcarbazone,  $\mathrm{CO}{<}\begin{matrix}\mathrm{NH.NHC_6H_5}\\ \mathrm{N:NC_6H_5}\end{matrix}$, the oxygen analogue of dithizone, gives a purple colour with mercury salts in neutral solutions, which can be compared with standards. Diphenylcarbazide, $\mathrm{CO(NH.NHC_6H_5)_2}$, the reagent used for chromium (p. 441), is sometimes recommended (Cazeneuve 1900), but the active agent is probably the carbazone which is readily formed from the carbazide by oxidation (Stock and Zimmermann 1928). For full development of the colour the solution must be neutral or slightly acid with acetic acid ; the optimum pH is about 4·0 (bromphenol blue). Small amounts of mineral acids or alkalies, and substantial amounts of nitrates, sulphates and ammonium salts affect appreciably the intensity of the colour. Several of the common metals also give red to purple colours with diphenylcarbazone in neutral solution (cf. Evans 1939). Moreover, the reaction fails in presence of hydrochloric acid or chlorides, owing to the extremely small degree of ionisation of mercuric chloride in solution. In this respect the reaction differs from that of mercury salts with the closely related compound diphenylthiocarbazone (dithizone), which is not inhibited by hydrochloric acid although it is so by hydriodic acid.

The diphenylcarbazone reaction will detect 0·0005 mg. of mercury in 2 ml. of solution, or 1 part in 4 million (Stock and Pohland 1926), but it is usually necessary to effect a previous separation of mercury from interfering substances either as sulphide or as metal.

The apparent absence of any reactive mercuric ions in a solution of mercuric chloride has been made the basis of a titration of chlorides with standard mercuric nitrate using diphenylcarbazone (or diphenylcarbazide) as indicator. The purple colour of the mercury compound does not appear until enough mercury has been added to combine with all the chlorine ions and free mercuric ions can exist in the solution (Dubský and Irtílek 1933). This titration is somewhat similar in principle to that of Evans' method (1939) for determining molybdic, tungstic, vanadic, phosphoric and arsenic acids by titration with lead nitrate solution in presence of an indicator containing diphenylcarbazone.

COLORIMETRIC DETERMINATION WITH *p*-DIMETHYLAMINO-BENZALRHODANINE. The formula of this substance is given on page 423. It gives a brick-red colour with small amounts of mercury ions. In neutral or acetic acid solution the colour is affected by the yellow colour of the reagent, and the reaction is best carried out in N/20 nitric acid solution in which this interference is very slight. Halides and sulphates must be absent. Other common metals do not interfere, with the exception of cuprous copper and silver.

Strafford and Wyatt have applied this method to the determination of mercury in grain. After destruction of organic matter with precautions against loss by volatilisation, mercury is precipitated as sulphide together with copper sulphide. It is then separated from copper by electrolysis, dissolved from the electrode with nitric acid and determined colorimetrically. Full details are given in Strafford and Wyatt's paper (1936). Even with this elaborate separation of mercury, silver, if present, may interfere, and a further purification of mercury, e.g. by volatilisation, may be necessary.

OTHER TESTS FOR MERCURY IN BIOLOGICAL MATERIALS. Several tests have been described for the examination of mercury-treated grain, etc. Some of them are variations of the well-known mercuric iodide test. If the seeds are extracted with hot dilute hydrochloric acid, the extract cooled and a strip of metallic silver immersed in it, any mercury present forms an amalgam on the surface of the silver which turns orange on being treated with a solution of potassium iodide (de Paolis 1931). Biancalini (1932) tests for the presence of mercury in urine by acidifying with sulphuric acid and heating on a steam bath with copper filings. The filings are dried, mercury distilled off in a small glass tube and the vapour brought into contact with cuprous iodide. A red double iodide of mercury and copper is formed. The test is stated to be sensitive to 0·005 mg. of mercury. Rangier and Rabussier (1935) determine mercury in biological material by adsorption on calcium phosphate, solution and reprecipitation as sulphide together with cadmium sulphide and eventual determination colorimetrically with potassium iodide and sodium hydroxide, cadmium hydroxide being removed by filtration. The yellow colour thus obtained is similar to that of the ordinary Nessler solution. The method is not very sensitive, being recommended for quantities of mercury between 0·1 and 1 mg.

The sky-blue insoluble double thiocyanate of mercury and cobalt is characteristic and has been used by Martini and Berisso (1934) for the detection of mercury in wine and in biological material. The test is not, however, very delicate, the limit being given as 0·08 per cent of mercury in solution.

Browning (1917) used a spectroscopic method for the detection of traces of mercury in toxicological work. The solution was first electrolysed with a gold or silver cathode and the cathode subjected to a spark discharge in an exhausted tube. With 10 ml. of solution it was possible to detect one part of mercury in 100 million parts of solution. Browning, in common with other investigators, draws attention to the loss of mercury by evaporation from very dilute neutral solutions of the chloride and also by adsorption on the walls of glass vessels (cf. also Stock 1939). Gold or silver foil, although purchased as pure, may contain traces of mercury from which it may be freed by heating for some time to 900°. It must also be borne in mind that many chemical reagents may contain minute amounts of mercury.

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[MOLYBDENUM]

THE metal molybdenum has recently attracted notice for two reasons : (i) its presence in minute amounts appears to be necessary for the fixation of nitrogen in the soil by *Azotobacter chroöcoccum* and similar organisms, and (ii) it is taken up by herbage from certain soils in quantities sufficient to cause serious poisoning in cattle and sheep.

DISTRIBUTION OF MOLYBDENUM. Ter Meulen (1932) drew attention to the almost universal presence of molybdenum in fertile soils and in plant and animal tissues. Soils contain 0·1 to 0·3 p.p.m. and in rare instances much more, cereals 0·2 to 0·6 p.p.m. and leguminous seeds 3 to 9 p.p.m., most of which is concentrated in the germ (Bertrand 1939). Molybdenum tends to accumulate in aquatic plants. Several animal tissues and biological fluids (blood, bile, milk and egg white) have been found to contain the metal in quantities of the order of 0·1 p.p.m. and liver about 1 p.p.m. (ter Meulen 1932).

EFFECT OF MOLYBDENUM ON NITROGEN FIXATION. Burk and Horner (1936) and Bortels (1936, 1939) find that molybdenum in concentrations of about 1 part in 50 million of a nutrient solution increases the fixation of nitrogen by *Azotobacter* as much as one hundredfold. In fact, if molybdenum is completely absent nitrogen fixation is inappreciable. It appears to be an essential element in the growth of the mould *Aspergillus niger*, but most metals seem at one time or another to have been claimed as indispensable nutrients for this mould : Steinberg (1937) suggests that it activates the reductase which makes nitrate nitrogen available for the synthesis of amino-acids and proteins. Brenchley and

Warington (1942) find that 0·1 p.p.m. of molybdenum in a nutrient solution stimulates the growth of lettuces, making them larger, deeper green and more resistant to disease, and Arnon and Stout (1939) find that 0·01 p.p.m. is essential for the growth of tomato seedlings. In pot tests with an Australian ironstone soil sodium molybdate at the rate of 2 lb. per acre doubled the yield of lucerne and made the foliage a darker green (Anderson 1942). Opinion at present seems to be that molybdenum is one of the few trace elements which must be considered essential for plant growth, and that the amount contained in plants is roughly of the order of 0·5 p.p.m. in the dry material.

MOLYBDENUM IN PASTURES AND ITS TOXICITY TO RUMINANTS. Certain pastures in Somerset and adjacent counties have been known for many years to cause scouring and loss of condition in ruminants. These are called locally ‘ teart ’ pastures (Gimingham 1910). Ferguson, Lewis and Watson (1940) found that this was due to the presence of relatively large amounts of molybdenum in the herbage, especially in clover. Beath and others (1935) had previously found in Wyoming that green food containing 89 p.p.m. of molybdenum when fed to livestock caused pathological changes similar to those caused by selenium. Teart pastures contain from 20 to 200 p.p.m. of molybdenum in the dry matter, whereas near-by pastures which are not teart contain not more than 5 p.p.m. Scott and Mitchell’s figures (1943) for pasture samples from north-east Scotland range from 0·22 to 3·81 p.p.m. in the dry matter. About 70 or 80 per cent of the molybdenum in teart pasture can be extracted with water. Experiments on sheep have shown that about 75 per cent of the amount ingested is excreted in the faeces and 25 per cent in the urine. Cows affected with scouring on such pastures can be quickly cured by moving them to other grazings low in molybdenum.

This action of molybdenum is strongly reminiscent of selenium poisoning (p. 222), but the latter is more severe and may affect all farm animals, whereas molybdenum seems to affect ruminants rather than horses or pigs (cf.

cobalt deficiency). Of the toxic elements comprised in the group arsenic, selenium, tellurium, molybdenum and vanadium, it is of interest that selenium and molybdenum are the only ones so far known to be taken up from soil by plants in amounts sufficient to make them toxic to animals. According to Franke and Moxon (p. 476) of these five elements arsenic is the least toxic to experimental rats.

Teart soils are nearly all derived from the clays and limestones of the Lower Lias and are calcareous and alkaline in character. They contain as much as 10 to 100 p.p.m. of molybdenum, equivalent to 20–200 lb. per acre in the top 9 inches of soil (Ferguson, Lewis and Watson 1940). Sulphate of ammonia reduces the alkalinity of the soil and also the amount of molybdenum taken up by the herbage, while basic slag and lime, which tend to raise the alkalinity, favour the uptake of molybdenum. This is partly due to the enhanced growth of clover on calcareous soils, clover being able to take up relatively large amounts of the metal.

Copper sulphate administered to cattle in doses of 1 to 2 g. daily prevents scouring on teart land. Possibly there may be some connection between copper deficiency and molybdenum poisoning. A similar condition marked by scouring has been observed by Brouwer and others (p. 9) in cattle grazed on reclaimed land in Holland and was attributed by them to lack of copper in the pastures. It could be prevented and cured by the administration of copper. Teart pastures in Somerset contain plenty of copper, 11 to 18 p.p.m. in the dry matter, and Brouwer has stated that the Dutch pastures in question do not contain molybdenum, or presumably not more than the traces normally present in plants (Ferguson and others 1940). It would seem, therefore, that the scouring of cattle in Somerset and in Holland, although characterised by somewhat similar symptoms and both amenable to copper treatment, must be due to different primary causes, unless we make the assumption, for which there is at present no evidence, that molybdenum is able to immobilise copper and interfere with its absorption from the alimentary tract, or with its functions after absorption, and that the symptoms are really those of copper deficiency. The blood copper in animals suffering from molybdenum poisoning is reported to be normal.

There is no indication, so far, that excessive amounts of molybdenum are taken up by table vegetables grown on Lower Lias soils, or that such vegetables have any appreciable toxic action on man.

MOLYBDENUM IN ANIMAL TISSUES. According to Bertrand (1942, 1943) the average amount of molybdenum in the dried tissue of invertebrates is 2 p.p.m. and in that of vertebrates 0·8 p.p.m. With mussels he found as much as 13·4 p.p.m. in the dried tissue. He has drawn attention to the copper-molybdenum ratio in animal tissues, and it is possible that this ratio in foods may be of significance in nutrition.

DETERMINATION OF MOLYBDENUM

THIOCYANATE METHOD. Molybdenum when present in traces in biological material is determined colorimetrically as the brownish-yellow thiocyanate, $Mo(SCN)_3$ (Stanfield 1935). This compound can be extracted with ether or cyclohexanol, giving a brownish-yellow solution which can be compared with standards.

The reduction of molybdenum to the tervalent state is effected with stannous chloride, which also reduces any iron present and prevents the formation of red ferric thiocyanate. Sandell (1936) found that molybdenum could be determined by this method in minerals without previous separation as sulphide, in amounts down to 1 part per million. Hurd and Allen (1935), comparing the colours in aqueous solution without ether extraction, determined the best conditions for colour development and stability, and found that unless the acidity of the solution is carefully controlled (about 5 per cent of hydrogen chloride) the colour is apt to fade rather quickly. Marmoy (1939) adapted the method, with ether extraction, to the determination of molybdenum in grass as follows.

2 g. of the dried sample are ashed at a low temperature.

Nitric acid should not be used, or molybdenum trioxide, MoO_3, may be formed which is volatile at temperatures below 450° (Niederl and Silbert 1929). The ash is extracted with 10 ml. of hydrochloric acid (sp. gr. 1·16) and the liquid diluted with water, boiled and filtered into a 100-ml. flask. After being cooled it is made up to the mark. A measured portion is diluted to 50 ml. and transferred to a separating funnel, hydrochloric acid being added until the solution contains 7 ml. of acid of sp. gr. 1·16. 3 ml. of a 10 per cent solution of potassium thiocyanate are added, followed by 3 ml. of a solution of 10 g. of crystallised stannous chloride in a mixture of 10 ml. of hydrochloric acid (sp. gr. 1·16) and 90 ml. of water. The liquid is well shaken between each addition. It is allowed to stand for 1 minute and then extracted successively with 10 ml. and 5 ml. of ether. A third extraction with 5 ml. of ether should yield a colourless solution, indicating that the extraction of the thiocyanate is complete. The depth of colour of the combined ether extracts is compared with that of a similar volume of ether to which is added the requisite amount of a standard solution of molybdenum thiocyanate in ether. If the colour of the ether extract fades, owing probably to the presence of peroxide, it can be restored and stabilised by shaking the ether extract again with stannous chloride and potassium thiocyanate solution.

To prepare the standard molybdenum solution 5 ml. of an ammonium molybdate solution containing 30 mg. of molybdenum in 1 litre are diluted and treated in a precisely similar manner with hydrochloric acid, thiocyanate, stannous chloride and ether, and the combined ether extracts run into a dry 25-ml. flask and made up to the mark with ether. The ether should have been previously shaken up with thiocyanate and stannous chloride. This ethereal solution contains 6 μg. of molybdenum per millilitre. Care is necessary to prevent changes in concentration of the standard solution as a result of evaporation. The solution is unstable and cannot be kept longer than a day. Cyclohexanol is claimed to be better than ether as a solvent as it is less volatile and the coloured compound is more readily extracted and is more stable (Hurd and Reynolds 1934).

AMMONIUM THIOMOLYBDATE METHOD. Ter Meulen (1925) found that the dark orange-red colour of ammonium thiomolybdate, $(NH_4)_2MoS_4$, allows of colorimetric titration. The solution containing a molybdate is treated with excess of ammonia and then with hydrogen sulphide. The reaction is slow, and hydrogen sulphide must be passed through the liquid for some time. The solution for comparison is prepared by treating a standard solution of ammonium molybdate in a similar way with ammonia and hydrogen sulphide. The method is stated to be quick and fairly accurate and not affected by alkalies. Vanadium and tungsten, if present, interfere. Bertrand (1939) gives a somewhat similar method which is claimed to be sensitive to 2 μg. of molybdenum in 100 g. of dry organic material.

DITHIOL TEST. Hamence (1940) finds that a solution containing molybdenum ions gives a brilliant yellow colour with thioglycollic acid and on addition of toluene 3:4-dithiol, $CH_3C_6H_3(SH)_2$ (cf. p. 156), a dark green precipitate. Tungsten gives a colourless solution and a light greenish-blue precipitate respectively. On addition of ammonia, molybdenum gives a bright blue solution and tungsten a colourless one. The limit of the test is 20 μg. of the metal in 10 ml. of solution.

SPECTROGRAPHIC DETERMINATION. This method, as developed by Scott and Mitchell for molybdenum, cobalt and nickel, is referred to on page 295.

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24

[VANADIUM]

VANADIUM possesses toxic properties comparable with those of arsenic, selenium, tellurium and molybdenum. It is a recognised industrial poison, and when inhaled as dust in factories its compounds cause irritation of the respiratory and digestive organs. The symptoms resemble in some respects those of acute arsenical poisoning. Daniel and Lillie (1938) found that doses of 2 to 4 mg. of vanadium, introduced directly into the stomach of rats in absence of food, quickly gave rise to symptoms of irritant poisoning with acute enteritis and that three daily doses of 2 mg. caused death. When mixed with rats' food 23 p.p.m. of vanadium in the form of sodium metavanadate (corresponding to about 0·25 mg. of vanadium daily) produced no symptoms of poisoning, but 90 p.p.m. (corresponding roughly to 1 mg. of vanadium daily) produced toxic symptoms. With 368 p.p.m. (3·7 mg. daily) the animals died. Taking the average weight of a rat as 200 g., this would suggest a toxic limit of between 3 and 5 mg. of vanadium per kilogram of body weight.

There appears to be no indication of chronic poisoning with repeated small doses of vanadium and the metal is rapidly eliminated. Proescher, Seil and Stillians (1917), administering vanadium to a human subject, found that about 88 per cent was excreted in the faeces and 12 per cent in the urine. The blood ash of rats receiving 0·25 mg. of vanadium in the daily food contained 35 to 40 p.p.m. (Daniel and others 1942).

The relative toxicity to rats of arsenic, selenium, tellurium, molybdenum and vanadium has been investigated by Franke and Moxon (1937). They fed rats on food containing

25 and 50 p.p.m. of these elements and found the relative toxicity to be as follows in decreasing order: selenium $>$ vanadium $>$ tellurium $>$ molybdenum $>$ arsenic. At concentrations of 50 p.p.m. in food, arsenic and molybdenum were slightly toxic, tellurium and vanadium moderately toxic and selenium very toxic.

VANADIUM IN SOILS, PLANTS AND LOWER ANIMALS. Most soils contain traces of vanadium; according to More (1943) the frequency of its occurrence in the earth's crust is about the same as that of nickel, copper, zinc and lead, but it is much more distributed. Occasionally it is present in fairly large amounts. Bertrand (1942) found 3·1 to 68 p.p.m. in twenty different soils, and Byers (1934) refers to a soil in the United States containing as much as 470 p.p.m. of V_2O_5. It also contained 2 p.p.m. of selenium. Wheat grown on this soil showed 25 p.p.m. of selenium and 9 p.p.m. of vanadium pentoxide, which indicates that vanadium can be taken up by plants but to a far less extent than selenium. It has been detected with the spectroscope in various species of grain in India (Boyd and De 1933), but no quantitative figures are given. Traces have been detected in sugar-beets (Kobert 1906). According to Vinogradov (1934) the vanadium in most terrestrial and marine plants and animals is of the order of 0·1 p.p.m. Bertrand (1941) gives a range of 0·27 to 4·2 p.p.m. with an average of 1 p.p.m. in the dried material of plants, and smaller amounts in the organs and tissues of vertebrate animals. In one fungus, *Amanita muscaria*, he found as much as 180 p.p.m. in the dry material. In small quantities vanadium stimulates plant growth and appears to resemble molybdenum in promoting growth and activation of *Azotobacter*. Basic slag, used as a fertiliser, may contain small amounts of vanadium up to 0·5 per cent.

A remarkable instance of the occurrence of vanadium in a definite biological rôle is provided by *Ascidia*, a group of marine worms. In these animals vanadium appears to take the place of iron or copper, and it has been suggested that the vanadium complex in the blood is able to take up and retain oxygen for use during the period that the animal is buried in tidal mud. Vinogradov (1934) found 0·03 per cent of vanadium in samples of marine mud and concluded that *Ascidia* can extract it and use it for the synthesis of a respiratory pigment. Webb (1939), on the other hand, finds that the vanadium chromogen is localised in a specialised cell and does not function as a respiratory pigment. He considers that the animal is able to extract it from sea-water, although this contains only 0·0003 p.p.m. (Goldschmidt).

VANADIUM IN HUMAN TEETH. Lowater and Murray (1937) found traces of vanadium in human teeth with the spectroscope and drew attention to the fact that vanadium is isomorphous with phosphorus and can replace it in the apatite molecule.

THE DETERMINATION OF VANADIUM IN FOOD

WITH AMMONIUM NITROSO-PHENYLHYDROXYLAMINE ('CUPFERRON'). The vanadium complex with cupferron is soluble in chloroform giving a yellow solution. According to Bertrand (1942) copper, iron, titanium and cerium also form complexes with cupferron soluble in chloroform and must first be removed. The ash of the sample after fusion with sodium carbonate is treated with cupferron and the solution extracted with chloroform under controlled conditions. The residue from the chloroform is subjected to wet oxidation, and iron (with titanium and cerium if present) is separated by precipitation with ammonia and hydrogen peroxide. Copper is then separated with hydrogen sulphide and vanadium in the filtrate brought into acetic acid solution. The solution is treated with cupferron and extracted with chloroform, and the vanadium in the extract determined colorimetrically.

Bertrand gives full details of the procedure, which he claims is capable of determining 2 μg. of vanadium. In a trial of the method with known amounts of vanadium (10 to 25 μg.) added to vegetable material, I found it difficult to recover more than half the vanadium added, and it appeared that further experience with, and probably modification of, the technique would be necessary before it could be depended upon for the small amounts of vanadium naturally present in foods. The chloroform extraction of the cupferron complex is best carried out on a solution containing about 3 per cent of acetic acid: a large excess of cupferron must be avoided. The colour in chloroform solution fades appreciably in 15 to 20 minutes so that matching should be done quickly. The limit for visual colorimetric comparison seems to be about 5 μg.

WITH TANNIN SOLUTION. Schoeller and Webb (1936) find that vanadium gives an intense blue-black colour or precipitate with a solution of tannin. This is not a specific test, but if vanadium is first separated from iron, etc., it is more sensitive than the cupferron test and will detect 2 μg. in 10 ml. of solution.

WITH HYDROGEN PEROXIDE. Vanadium gives a red colour with hydrogen peroxide similar to that given by titanium, sensitive to 1 part in 160,000 (Meyer and Pawletta 1926). In presence of sodium fluoride the reaction with titanium is suppressed to an extent which makes it possible to determine vanadium with a fair degree of accuracy, but under these conditions the delicacy of the vanadium reaction suffers somewhat, and the method is hardly sensitive enough for the examination of plant material (Bertrand 1942).

OTHER COLORIMETRIC METHODS. Wright and Mellon (1937) determine vanadium by the yellow or brownish colour given with sodium tungstate and phosphoric acid. Vanadium also combines with 8-hydroxyquinoline to form a compound soluble in *iso*butyl or *iso*amyl alcohol to a plum-coloured solution which can be compared with standards (Bach and Trelles 1940).

Vaughan (1942) determines quantities of vanadium down to 5 μg. in 10 mg. of steel by the violet oxidation product produced with strychnine in concentrated sulphuric acid. The optical density of the coloured solution is determined in a Spekker photo-electric absorptiometer.

SPECTROGRAPHIC DETERMINATION. Daniel, Hewston and Kies (1942) determine vanadium spectrographically with a graphite arc, with addition of chromium to serve as an internal standard. The intensity of the vanadium lines 3183, 3184 and 3185 is compared with that of the chromium line 3188. According to Bertrand (1942) graphite always contains traces of vanadium, and moreover the spectral lines are greatly intensified in presence of alkaline salts which makes the method unsuitable for small quantities of vanadium in foods. The spark discharge from fused alkaline carbonate in a small platinum crucible is free from these disadvantages and can be used for the approximate determination of 0·1 to 0·2 p.p.m. of vanadium in vegetable tissues.

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25

[TITANIUM]

TITANIUM is widely distributed in soils and is frequently present in very small quantities in food. Soils may contain up to 1 per cent of titanium dioxide, the usual amount being 0·3 to 0·6 per cent, and the ash of plants from nil to 0·27 per cent (Geilmann 1920). Griffiths-Jones (1923) found 1·3 to 2·5 per cent of titanium dioxide in various samples of Nile silt and 0·4 per cent in the ash of wheat grown on these soils. New Zealand pastures were found by Askew (1930) to contain from 10 to 85 p.p.m. of titanium in the dry matter. Bertrand (1929) gives 0·1 to 5 p.p.m. and Kaminskaya (1937) 1 to 10 p.p.m. for various plants.

According to Maillard and Ettori (1936) the organs of the human body contain from 0·015 to 0·11 p.p.m. of titanium ; in blood they found 0·03 p.p.m. Tschuiko and Voinar (1939) found from 0·02 to 0·3 p.p.m. in dogs' livers and smaller amounts in the other organs. The hair contained as much as 2·8 p.p.m.

Sodium titanate has been found to stimulate the growth of mustard, pea and lucerne crops by as much as 30 to 40 per cent, titanium apparently replacing iron in the plants ; the iron content decreases with increasing uptake of titanium (Nemec and Kás 1923). Presumably, if this replacement of iron were carried too far, growth would be interfered with. There is no indication that titanium has any physiological function in the human body and it is reputed to be non-toxic.

Titanium dioxide is often used as an opacifier in enamels in place of tin. It is also sometimes used instead of white lead in paints which may come into contact with food, e.g. for painting the ends of cider barrels and the inside of fish boxes.

DETERMINATION OF TITANIUM. Small amounts of titanium are usually determined colorimetrically by means of the yellow colour given with hydrogen peroxide in dilute sulphuric acid solution. In Griffiths-Jones' method (1923) the sulphated ash of the material after elimination of silica and fluorine, if these are present, is fused with about fifteen times its weight of potassium hydrogen sulphate, the fused mass dissolved in warm 5 per cent sulphuric acid solution and the solution cooled and made up to 1 litre. 25 ml. of this solution are transferred to a Nessler glass, 5 ml. of 10-volume hydrogen peroxide added, the whole made up to 50 ml. and the colour matched with a standard solution of titanium. The standard solution is prepared by fusing 0·2 g. of titanic acid with potassium hydrogen sulphate, dissolving the fused mass in 5 per cent sulphuric acid, as with the sample, and diluting the solution to 1 litre. 1 ml. of this standard solution contains 0·2 mg. of titanium dioxide.

If vanadium happens to be present in the sample it will give a brownish-yellow colour with hydrogen peroxide and interfere with the determination. Bromides also interfere since they give a yellow colour with hydrogen peroxide (Lewis 1936).

Maillard and Ettori's method (1936) for determining traces of titanium in tissues is as follows. It is based on the work of Thornton (1914) and of Thornton and Hayden (1914) on the separation of titanium and zirconium from iron. The material is ashed, the ash dissolved in 5 per cent sulphuric acid solution and a little ferric iron added if necessary. Tartaric acid is added and then a solution of the ammonium salt of nitroso-phenylhydroxylamine (' cupferron ') which precipitates iron, titanium and a little copper, leaving any phosphoric acid in solution. The precipitate is washed and calcined, the oxides dissolved in 5 per cent sulphuric acid, tartaric acid added and the liquid neutralised with ammonia and then made faintly acid with sulphuric acid. Iron is now precipitated with hydrogen sulphide and removed by filtration, leaving titanium in solution. More sulphuric acid is added and the solution heated to drive off hydrogen sulphide.

The presence of ammonium sulphate and tartaric acid prevents sufficient concentration of the solution at this stage, and Maillard and Ettori therefore add several milligrams of zirconium sulphate and re-precipitate titanium together with zirconium by a second addition of cupferron. After incineration the precipitate contains only titanium and zirconium. It is dissolved in a few drops of sulphuric acid, 0·3 ml. of perhydrol added, the solution made up to 10 ml. and the titanium determined colorimetrically. Zirconium does not interfere. The sensitivity of the method is given as 0·1 μg.

Dihydroxymaleic acid (p. 373) was suggested by Fenton as a still more delicate reagent than hydrogen peroxide for traces of titanium.

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26

[INDIUM]

INDIUM is a soft untarnishable metal melting at 176°, similar in many of its chemical characteristics to aluminium. Owing to the discovery of improved methods of extracting it from zinc ores it has in recent years fallen in price from about £600 to about £3 or £4 per oz. and is being used for plating copper, silver and various other metals and alloys. It is claimed to be particularly useful in preventing corrosion of cadmium alloy bearings by lubricants containing organic acids. For this purpose the process differs from true plating in that the metal, after electrodeposition of a film of indium at the rate of about 5 mg. per square inch of surface (McCord and others 1942), is then heat-treated, with the result that the indium diffuses into the outer layer to a depth of 0·01 to 0·015 inch, forming an alloy of low indium content which is resistant to corrosion. At one time it was thought that silver-ware could thus be prevented from tarnishing without affecting its appearance or properties, but Raub and Schall (1938) find that indium-silver alloys tarnish just as readily as silver. A plated coating of indium does protect silver from tarnishing, but its colour is not that of silver and it is too soft for ordinary domestic use.

It is possible that indium may be used, like cadmium (p. 428), for plating articles such as refrigerator trays which may come into contact with food. In view of the unfortunate results which followed the use of cadmium for this purpose without sufficient inquiry into its toxic properties, it is satisfactory to find that the hygienic aspects of indium plating have already been the subject of some investigation.

Harrold and others (1943) immersed pieces of indium-plated silver sheet in various foods under sterile conditions for about six months and found that with tomatoes, apple juice, sour cherries and vinegar practically all the indium had been dissolved from the silver at the end of this time. With sweet corn there was no solution of indium. Experiments with shorter times of contact were not carried out.

In feeding experiments on rats (averaging 170 g. in weight) it was found that daily doses of 5 and 10 mg. of indium sulphate, continued for 27 days, had no ill effects. With daily doses of 20 and 30 mg. for a period of 72 days the rats receiving the 30 mg. doses lost weight during the latter part of the period, became inactive and had rough coats. Post-mortem examination showed no definite changes. Indium is much more toxic when injected either subcutaneously or intravenously (von Oettingen 1932, McCord and others 1942), and its comparative non-toxicity in food may perhaps be ascribed to relatively low absorption from the digestive tract, in which respect it seems to resemble aluminium (Steidle 1933). Its toxicity, and its behaviour in contact with foods, require further investigation before it is used for plating food receptacles.

DETERMINATION OF INDIUM IN FOOD, ETC. Harrold and others (1943) destroy organic matter by heating with nitric acid and hydrochloric acid and take up the residue with 50 ml. of water. The acidity is adjusted to pH 3·5 and the solution thoroughly extracted with a strong solution of dithizone in chloroform. This removes copper, mercury and possibly zinc and bismuth, silver having already been immobilised as chloride. The dithizone-chloroform extract is rejected and the aqueous solution adjusted to pH 9·5. Ammonium citrate is added to prevent interference by ferric hydroxide or alkaline earth phosphates, and the solution is again thoroughly extracted with dithizone which removes practically all the remaining metals (p. 131) except tervalent ones with which dithizone does not react. This dithizone-chloroform extract is rejected. Indium being a tervalent metal remains in the aqueous solution. 15 ml. of water containing 0·03 g. of ammonium citrate, 0·04 g. of potassium cyanide and 1·5 ml. of 0·880 ammonia are added, followed by 2 drops of concentrated hydroxylamine hydrochloride solution. This apparently reduces tervalent indium to a lower state of oxidation in which it can combine with dithizone. The presence of cyanide ensures the retention of traces of copper, zinc, etc., which may have escaped the previous extractions. The solution is now adjusted to pH 7·0 and extracted five times with 5 to 10 ml. of a strong solution of dithizone in chloroform. During these extractions the solution is gradually brought slightly on the acid side of neutrality, which is found to facilitate the extraction of indium. Eventually the combined dithizone extracts are shaken with dilute acid which decomposes the indium-dithizone complex and extracts the metal from the chloroform. The solution is evaporated to a small volume and indium determined colorimetrically with quinalizarin as follows.

The solution is transferred to a 15-ml. centrifuge tube, diluted with water and ammonium chloride added. A solution of quinalizarin in 0·880 ammonia is added and the contents of the tube allowed to stand and then centrifuged. The colour of the supernatant liquid is compared with that of indium standards similarly treated. It is claimed that this method is suitable for amounts of indium from 1 to 25 μg., but the details of the dithizone extraction as given are by no means clear and several points would seem to require elucidation.

Indium may also be determined colorimetrically with 8-hydroxyquinoline (Moeller 1943). Within a pH range of 3·2 to 4·5 it is completely extracted from solution by shaking with a chloroform solution of 8-hydroxyquinoline, as the bright yellow compound $In(C_9H_6ON)_3$. A distinct colour is given with 1 mg. of indium in 1 litre of solvent. The hydroxyquinoline compounds of aluminium, tin (stannous), bismuth, iron (ferric and ferrous), cobalt, nickel, copper, gallium and thallium are stated by Moeller to be extracted by chloroform from solutions at pH 3·5 and to interfere with the determination. Indium must therefore first be separated from these metals.

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27

BARIUM AND STRONTIUM

BARIUM has been shown by Seaber (1933) to occur in Brazil nuts in amounts of the order of 0·1 to 0·3 per cent. This seems to be the only instance of its natural occurrence in food in considerable amounts. Hinks (1911) found that Gorgonzola cheeses are frequently coated with a mixture of barium sulphate (barytes) and tallow, the coating constituting 16 to 27 per cent of the weight of the cheese. In some instances the coating had been forced into the holes in the cheese, with the result that plugs of barytes were found running into the cheese up to $\frac{5}{8}$ inch beyond the inner surface of the rind, which might well lead to the eating of fairly large amounts of barium sulphate. Pure barium sulphate being insoluble is probably non-toxic (cf. barium meals in radiography) but contamination with soluble barium compounds is a possibility. The toxic dose of soluble barium is stated to be of the order of 0·2 g.

About the year 1887 the barium saccharate process was introduced in beet sugar refineries on the Continent, but was soon abandoned owing to the danger of poisoning from residual barium in the various sugar products. Browne, in his *Handbook of Sugar Analysis* (1912), says that the barium process was still used in Italy at that time.

Barium fluosilicate, $BaSiF_6$, is often used as an agricultural insecticide. When diluted with calcium hydroxide, clay, flour or talc, it is stated to be better than lead arsenate for the control of cabbage caterpillars (Diamond 1935). In the form of a 10 per cent dust it has been used against caterpillars on vines. A 20 per cent dust causes burning of the foliage. Barium fluosilicate has been widely used in the

United States and in France against the Colorado beetle on potato haulms. Retention of the powder by food going into consumption would be objectionable on the score both of barium and of fluorine, although possibly not so objectionable as lead arsenate.

According to Timm (1932) minute traces of barium occur in animal bones as a natural constituent, but Kunowski (1932) found no barium in normal human bones with the spectroscope (cf. also Voinar and Lazovskaja 1942).

Strontium was found by Gerlach and Müller (1934) in animal tissues to the extent of 0·01 to 0·1 p.p.m. Lundegårdh, using a spectroscopic method, found 0·1 to 0·3 p.p.m. in a number of human livers, the corresponding calcium content being 30 to 40 p.p.m. Strontium may occur in small amounts in beet molasses treated by the strontium method for sugar separation, and is also sometimes present with barium sulphate in the coating of Gorgonzola cheese. It occurs together with barium in certain natural brine waters (Francis 1923). Sea-water contains 10 p.p.m. of strontium but only 0·05 p.p.m. of barium (Goldschmidt 1937).

Experimentally, strontium in relatively large doses can produce rickets in animals owing to the formation of an insoluble phosphate which is not absorbed but is excreted in the faeces (cf. beryllium). In other respects strontium salts are only feebly toxic. Strontium may be retained to some extent in the body and deposited in the bones (Fay and others 1942).

Strontium and barium in small amounts stimulate the growth of plants, but in larger amounts they inhibit growth according to the extent to which they replace calcium (Scharrer and Schropp 1937).

A method for the determination of barium and strontium in presence of excess of calcium is given by Francis (1923) and of barium in Brazil nuts by Seaber (1933). Barium and strontium salts can also be detected in presence of calcium salts in neutral solution by the brown-red colour given with sodium rhodizonate, but several other bivalent metals give a similar reaction (Feigl 1943).

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28

LITHIUM

AND OTHER METALS

LITHIUM and a few of the rarer metals are of some interest in connection with food.

According to Goldschmidt (1937) the average amounts of lithium, rubidium and caesium in the rocks of the earth's crust and in sea-water are as follows :

	Earth's crust	*Sea-water*
Lithium . . .	65 p.p.m.	0·11 p.p.m.
Rubidium . . .	310 p.p.m.	0·2 p.p.m.
Caesium . . .	7 p.p.m.	0·002 p.p.m.

Rubidium and caesium are taken up by plants almost as quickly as potassium (Collander 1941) but cannot replace potassium to any great extent without toxic effects (Alten and Gottwick 1933). With oats in sand culture the ratio of rubidium to potassium in the nutrient solution may be as much as 1 to 7 before serious injury takes place (Blanck and others 1933).

Lithium occurs spasmodically in traces in foods and rubidium is fairly widely distributed (Sheldon and Ramage 1931). According to Kent and McCance (1941) the daily intake of lithium in food is about 2 mg., or roughly the same as that of copper ; of this one-third to one-half is absorbed and excreted in the urine. A sample of brown bread contained 4·2 p.p.m. and white bread 1·7 p.p.m. Lundegårdh's figures for lithium in the livers of hospital patients range from 0·3 to 9 p.p.m.

Rubidium is more abundant. Burkser and others (1930, 1932) found 3·4 p.p.m. in beans and 5·7 p.p.m. in barley, and Lundegårdh (1934) found an average of as much as 14 p.p.m. (calculated on the fresh substance) in human livers from hospital patients. According to Follis (1943) rubidium, and to a smaller degree caesium, can partially replace potassium in the human diet, but excess of either is toxic.

Traces of lithium can be precipitated as lithium potassium ferric periodate and the iron in the precipitate determined colorimetrically. Calcium, magnesium and sodium must be absent. Sodium is separated by extraction of the mixed chlorides with ether-alcohol mixture which dissolves lithium chloride (Sandell 1944). Kent (1940) has described a spectrographic method for use in investigations on the effect of lithium on plants, and Lundegårdh also used the spectrograph for his investigations on human livers. Robinson (1918) gives a method for the approximate determination of rubidium and caesium in plant ash, depending on their separation as platinichlorides and eventual comparison of the intensity of the spectra with standards. Burkser determined rubidium in plant material by precipitation as the double rubidium tin chloride Rb_2SnCl_6.

Beryllium salts, when fed to rats, poultry, etc., produce the so-called beryllium rickets, due to immobilisation of phosphorus by formation of insoluble beryllium phosphate (Branion and others 1939, Guyatt and others 1933, Jacobsen 1933, Kay and Skill 1934). Various alloys of beryllium with silver and other metals have been suggested for plant and vessels in contact with foods, being resistant to corrosion by vinegar, fruit and vegetables. The mere traces of the metal which might thus gain access to food would be far smaller than the amount required to produce rickets in animals, and the proportion of phosphorus immobilised would be negligible. The biochemistry and industrial toxicology of beryllium are the subject of a comprehensive report by Hyslop and others (1943).

Zirconium and cerium oxides are frequently used as opacifiers in hollow-ware enamels, and insignificant traces may occasionally find their way into food from this source.

Germanium is taken up from some soils by cereals, especially oats (Geilmann and Brünger 1935). This metal and also gallium and scandium have recently been shown to occur much more widely in rocks and soils than had hitherto been suspected. Gallium is present in all aluminium minerals to the extent of 0·005 to 0·01 per cent of the aluminium and is 150 times more abundant than silver (Goldschmidt 1937). This does not, of course, necessarily imply that it is taken up by plants ; for instance, aluminium, in spite of its being the commonest metal in soils, is practically absent from plants. On the other hand, the occurrence of certain rare elements in forest mould and in coal ash indicates that they must be taken up by plants and thus become concentrated in decaying vegetable matter (Goldschmidt 1937, Davies and Morgan 1938, Babička 1943). Oak and beech humus in one locality in Germany contains as much as 70 p.p.m. of germanium.

Germanic acid resembles boric acid in forming acid compounds with polyhydroxy alcohols and also in giving the quinalizarin reaction (Poluektoff 1935). Methods for its determination have been given by Davies and Morgan (1938). According to Schwarz and Scholz (1941) it is non-toxic when ingested.

Rare earth oxides can partially replace calcium in the growing plant according to Robinson and others (1938), who state that they found 0·2 per cent of these oxides in the dry leaves of a hickory tree growing on a vein of pegmatite. Mitchell's figures (1940) for the occurrence of rare earth oxides in Scottish soils may be noted. It is doubtful whether these can be taken up in significant amounts by food plants, although they have been stated to accumulate in certain plants, notably lupins, beet and maple leaves. Drobkov (1937) maintains that small amounts are essential for the normal development of pea plants. According to Lux (1938) bone-ash contains about 0·004 p.p.m. of rare earths.

Tungsten resembles molybdenum in many respects and is toxic to animals, but has not been recorded as occurring in vegetation or in food, although it is said to stimulate early growth in plants (Bortels 1936). Kinard (1941) has studied its toxicity to rats and has given a method for its determination in biological material (Aull and Kinard 1940). Tungstic acid solutions, as is well known, give a deep blue colloidal solution when reduced. In presence of potassium thiocyanate the colour on reduction is blue in neutral or alkaline solution but green or yellow in acid solution according to the conditions of experiment. With titanium chloride in a solution containing thiocyanate and strongly acidified with hydrochloric acid tungstic acid gives a yellow colour which is suitable for colorimetric determination. The smallest amount of tungsten thus determined by Aull and Kinard is 0·5 mg. in 10 g. of sample. Hamence's dithiol test for molybdenum and tungsten is referred to on page 474. Miller (1944) has extended Hamence's test to the detection of tungsten in metallurgical work and finds that in presence of concentrated hydrochloric acid and *iso*amyl acetate, dithiol gives a characteristic blue colour with as little as 2 μg. of tungsten. The test would appear to be of wide application.

Thallium does not occur naturally in plant or animal tissues, but in view of its increasing use as a vermin poison it may occasionally gain access to foods. Grain treated with thallous sulphate, which is tasteless and odourless, is sometimes used for killing rats, and poultry and game poisoned by such grain might conceivably be offered for human consumption. The lethal dose for mammals is about 25 mg. per kilogram, which would correspond to about 3 to 6 mg. for a rat. For game birds it is 12 to 30 mg. (Shaw 1933). Shaw suggests that game poisoned with thallium would probably be innocuous to man, but there is no definite information on this point.

Thallium is eliminated from the body very slowly and its action is cumulative. One of its most characteristic effects in large doses is loss of hair. In small doses it interferes with reproduction in rats, and even in infinitesimal amounts it causes slight degenerative changes in the brain (Roche Lynch and Scovell 1930). It is very poisonous to plant life in concentrations greater than about 5 p.p.m. in soil, and soluble thallium salts are retained obstinately by soil through several seasons (Crafts 1934). Like most other toxic metals, it has a stimulating effect in very minute quantities (Horn and others 1936). Richards (1932) found that traces of thallium (1 p.p.m. in the nutrient solution) increase the yield of yeast by as much as 80 per cent. Larger amounts are toxic. He suggested that thallium might occasionally be found as an ingredient of commercial yeast stimulants and emphasised that it should never be used when the yeast is intended for human food.

Thallium is readily detected with the spectroscope, being characterised by a strong green line at wave-length 535·1 mμ. It is determined in toxicological work by precipitation as yellow thallous iodide which is dried at 120° and weighed (Roche Lynch and Scovell 1930) or titrated (Samaan and Mikhail 1943).

Haddock (1935) has reviewed the analytical chemistry of thallium and gives a method for the determination of small quantities (5 to 200 μg.) in metals. The principles of the method are equally applicable to determination in foods or biological material. Thallium is first extracted, in the reduced state, with dithizone in chloroform from a faintly alkaline solution containing cyanide and ammonium citrate. After evaporation of the chloroform and destruction of organic matter, thallium in the residue is oxidised to thallic chloride with bromine in presence of ammonium chloride. Excess of bromine is removed by boiling and by addition of a small quantity of phenol solution. Potassium iodide is now added, which reacts with thallic chloride, and the iodine set free is determined colorimetrically with starch-glycerol solution. Full details are given in Haddock's paper. Gettler and Weiss (1943) have reviewed the qualitative tests for thallium in biological material.

Uranium has been the subject of soil experiments and is stated to increase wheat yields when present in soil in amounts up to 170 p.p.m., but higher proportions are injurious and anything over 1,700 p.p.m. kills wheat seedlings (Demoussy 1932). It is credited with being one of the most toxic of all metals to animal life, particularly when its compounds are inhaled, and causes severe nephritis. Hoffmann (1942, 1943), as a result of fluorescence measurements, claims to have established the presence of traces of uranium, of the order of 0·1 μg. per kilogram, in various foods and rather larger amounts in human and animal glands and in bone ash, but the measurements are liable to be vitiated by traces of zinc. Uranium is unlikely to occur in foods in significant amounts, but the possibility of highly toxic metals being used as vermin poisons or as seed disinfectants and thus finding their way accidentally into food should always be borne in mind.

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DATE OF ISSUE

18 AUG 1949

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